Chapter One: Introduction

Chapter Overview

This chapter contains the following topics:

- How to Use the Leica FW4000 User Manual
- Style Reference
- System Hardware Configuration
- Software Installation
- Help
- Using Leica FW4000
- Preparing Your Hardware and Samples
- Using Leica FW4000 in a Network Environment
  - Configuring Leica FW4000 Stations in a Network
- Automatic Calibration

How to Use the Leica FW4000 User Manual

This manual is part of the **Leica FW4000** User Manual and comprises two discrete areas containing information targeted at different user competencies. You can either:

- Use the Quick Start (Chapter 3) walkthrough of the major elements of the end-to-end process. **Leica FW4000** has an easy-to-use and intuitive interface; therefore, the walkthrough is simple and matches the design features built into the application software.
- Perform experiments by using Chapters 4 to 12 sequentially. There is one chapter for each of the major steps required to perform an experiment, followed by a specific chapter covering the image viewer.

  When starting to perform an experiment, you are recommended to start at Chapter 4. Then work through the User Manual until you complete the experiment, or arrive at a natural point to pause. When you restart, dip into the appropriate chapter and continue working through the process.

  You could run a complex and lengthy image capturing sequence, that does not require your attendance, overnight.

Style Reference

The following conventions have been used to make this manual easier to read:

Menus	**FILE** (bold, capitals).
Menu Commands/ Buttons/ Dialogs	**Open, OK** (bold).
References	*Chapter 1* (italics).
Notes, Tips, Warnings, References and Instructions	Appear as follows and contain additional information

Note: Additional information that should be noted.

Refer to: References to other relevant material.

Tip: Tips to help you use the program more productively.

WARNING: Important information to which you should pay careful attention.

One Step instructions: Operations that can be performed in a single step.

Step-by-step instructions: Numbered instructional steps.

Information that only applies in certain situations is enclosed in boxes.

System Hardware Configuration

The hardware configuration is described in the **Leica FW4000** Release Notes. This document also contains important information concerning the installation of the hardware.

Refer to: The *Leica FW4000 Release Notes* for information on the required hardware configuration.

Software Installation

Leica FW4000 is provided on a CD-ROM, and must be installed onto your hard disk before it can be run.

To install Leica FW4000 from the CD-ROM:

1. Ensure that Windows is running.
2. Insert the CD into your CD-ROM drive.
3. The **Leica FW4000** Installation dialog appears.

Note: The options available in this dialog may vary, depending on your licence agreement.

4. Ensure that the **Leica FW4000** checkbox is ticked.
5. Check the **Leica Deblur** checkbox if you want to install this component and you are licensed to use **Leica Deblur and 3D Visualise**. This allows you to perform **Blind Deconvolution** and **3D Visualise** operations. You need the correct dongle for this component to be available. It does not work in demo mode.
6. When the installation has finished, click **OK** to close the

Installation dialog.

7. Restart your computer.
8. Launch the **Leica FW4000** application either by clicking on the desktop icon or by selecting it from the **START** menu.

Help

Use the standard customised **Leica FW4000** Help by clicking **HELP** in the main menu.

Using Leica FW4000

Leica FW4000 has an easy-to-use and intuitive interface. The Microsoft® Windows™ environment allows you to perform tasks quickly and easily.

The **Quick Start** option in Chapter 3 helps you to familiarise yourself with the structure, operation and principles of the program.

Refer to: *Chapter 3* for information on running Quick Start.

Preparing Your Hardware and Samples

To prepare your hardware:

1. Ensure that your microscope and fluorescent lamp power supply is switched on. If it is an automated microscope, you will need to wait until it is initialised before starting **Leica FW4000**.
2. Turn on your workstation and start **Leica FW4000**.
3. Turn on the camera power supply, if applicable.
4. Ensure that the fluorescent lamp is working properly, and is correctly centred and working at maximum fluorescent capability.
5. Check the appropriate filter cubes that visualise each individual fluorochrome are fitted.
6. Check that the connection between your workstation and your microscope is working correctly.

Preparing a sample:

1. Take a sample and visualise it with a high-power, oil immersion objective. The type of sample depends on the type of analysis you are performing.
2. When you have a suitable specimen, pull out the light path bar to direct all the light to the camera, and then close the shutter.

Using Leica FW4000 in a Network Environment

You can network a number of **Leica FW4000** workstations in order to access experiments captured on one station over the network on an additional workstation. Checks have been added to the software to handle the following scenarios:

- Access of the same experiment by multiple users on a network
- Access of the same experiment by one user on different stations in the network

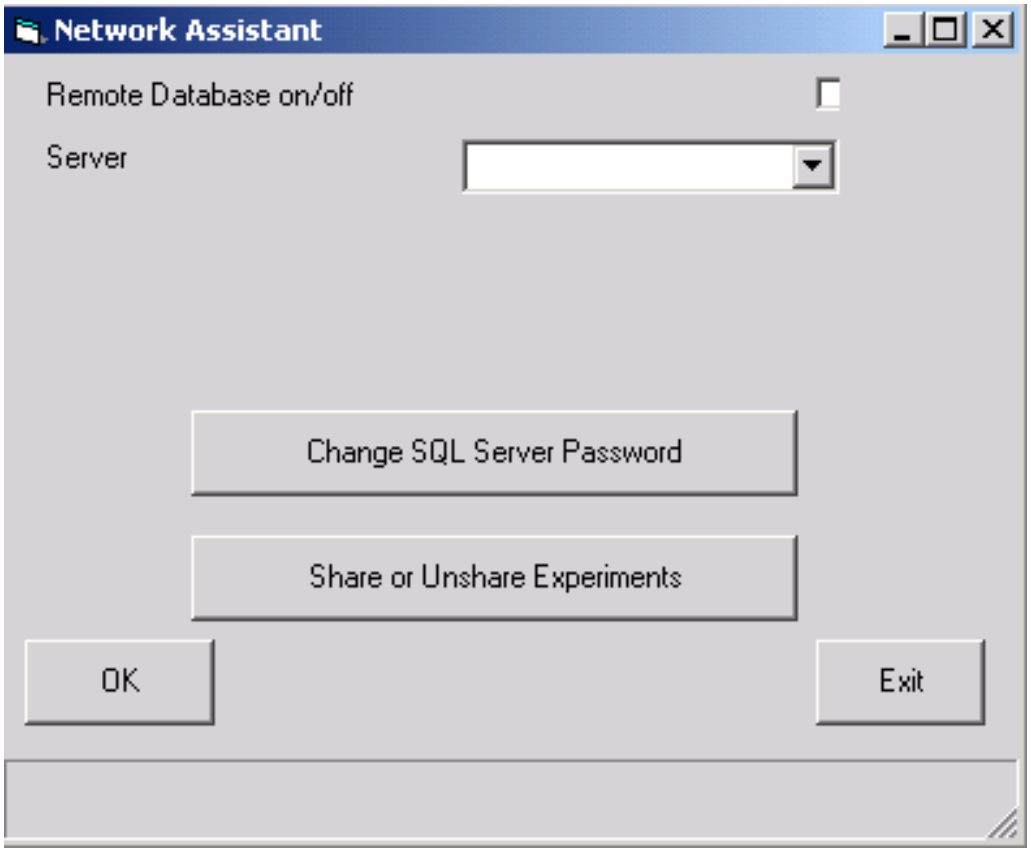
Leica FW4000 installation normally saves images in the local file system, and information about the images and all experimental data in a local SQL server database. **Leica FW4000** connects to the database by using information stored under a named system data set. You can change this information so that the data source is another SQL server somewhere else on the network. Images and other data are stored in the file system at locations you determine when an experiment is created. If you place this data on a network drive that is mapped to a drive letter, then other users can access this data and the database will record information about these files using the mapped drive letter, so that **Leica FW4000** will behave normally.

Configuring Leica FW4000 Stations in a Network

Each workstation in the network must map each network drive to be accessed. If one workstation is assigned to act as the server, this workstation must also map the network drive on which the database resides as the same letter drive as all other workstations in the network.

To set up network working:

1. Run the Network Assistant Application. This application needs to be run on each workstation in the network and must be run on the **serve**r workstation **first**.
2. Launch the **Network Assistant** application by browsing to: C:\Program Files\LeicaFW4000\NetworkAssistant.exe

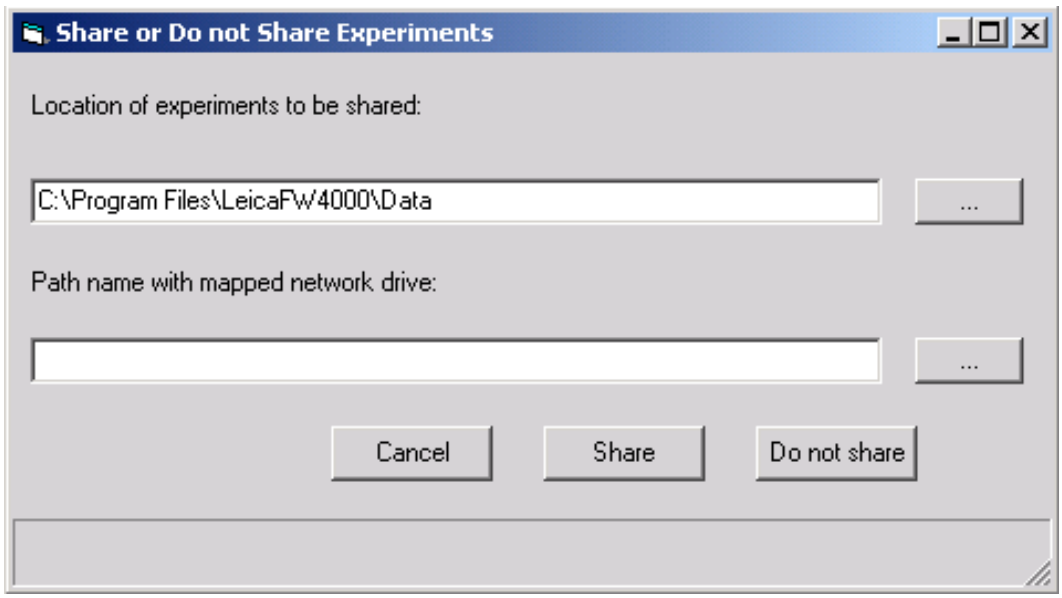


3. Define where your data is to be stored by:
    - Checking the **Remote Database on/off** box, to hold the database for the workstation on a remote workstation (for example, one of the workstations in the network will be treated as the server), or
    - Unchecking the **Remote Database on/off'** box, to hold the database for the workstation on the workstation (for example, the workstation will act as the server).
4. Type the name of the database server in the **Server** text field for the remote workstations (only).

Note: Available server names are shown in the drop-down list. This is usually the name of the workstation as it appears to the others on the network, but may be something different.

Note: The workstation assigned as the server must be switched on and fully operational, with the SQL server running for the network set-up to work.

5. To share experiments which were created when the system was not networked, but which are now on a mapped network drive:
    - Click Share or Unshare experiments and type the mapped path of the relevant experiments
    - Click Share.



Automatic Calibration

The FW4000 can run in combination with an automated Leica microscope or with a manual microscope. For either combination, the system can calibrate itself using information that is read automatically or entered by the user.

The following information is required for automatic calibration:

- CCD pixel size. This is entered automatically by the software, if a Leica DC, DFC or camera is connected
- C mount magnification. This is entered by the user
- Magnification data. This is defined by the objective and magnification changer, which can either be read automatically from an automated microscope or entered manually by the user. Both of these are set at the time the experiment is performed

To use automatic calibration:

1. From the hardware setup menu, select Optics Data.
2. Click on the Lens Data tab.
3. Either:
   - The objectives listed in the Lens Data tab will be filled in automatically if you are connected to a DM microscope
   - Manually complete the Lens Data tab

Refer to: ***Leica FW4000*** *Release Notes* for more information on lens data.

6. Click on the Calibration tab.
7. Check the Automatic calibration check box. The software will take the CCD pixel size and the C mount magnification to calculate the distance between two pixels for each objective in the list.

Note: The calibration factors displayed will change depending on the magnification changer that is currently selected for the experiment. Objective and magnification changers are set in the Live dialog in the Advanced tab. If your experiment was performed with the wrong objective recorded, you can change it afterwards in the Lab Book.

Manually calibrating the system for a given objective

A user may decide to manually calibrate the system for a given objective.

Information about the nature of the calibration is recorded in the user column of the Calibration tab:

- (Calculated) means the calibration factor was calculated automatically by the system or from a user-defined calibration
- (FW4000) means the calibration factor was manually defined by a user

If:

- You calibrate the system manually, the calibration for all other objectives will be recalculated using the measured calibration as the basis for all other calculations
- You calibrate more than one objective manually, the FW4000 will use the first objective calibrated as the basis for all other derived calibration values

➡ To delete all manual calibrations, click Clear All calibrations.

1 2 3 After you have defined a calibration factor manually, you must reset the calibration values in the table to 'derived':

1. Uncheck and check again the automatic calibration box.
2. Click Apply.

Chapter Two: Administration Tools

Chapter Overview

This chapter contains the following topics:

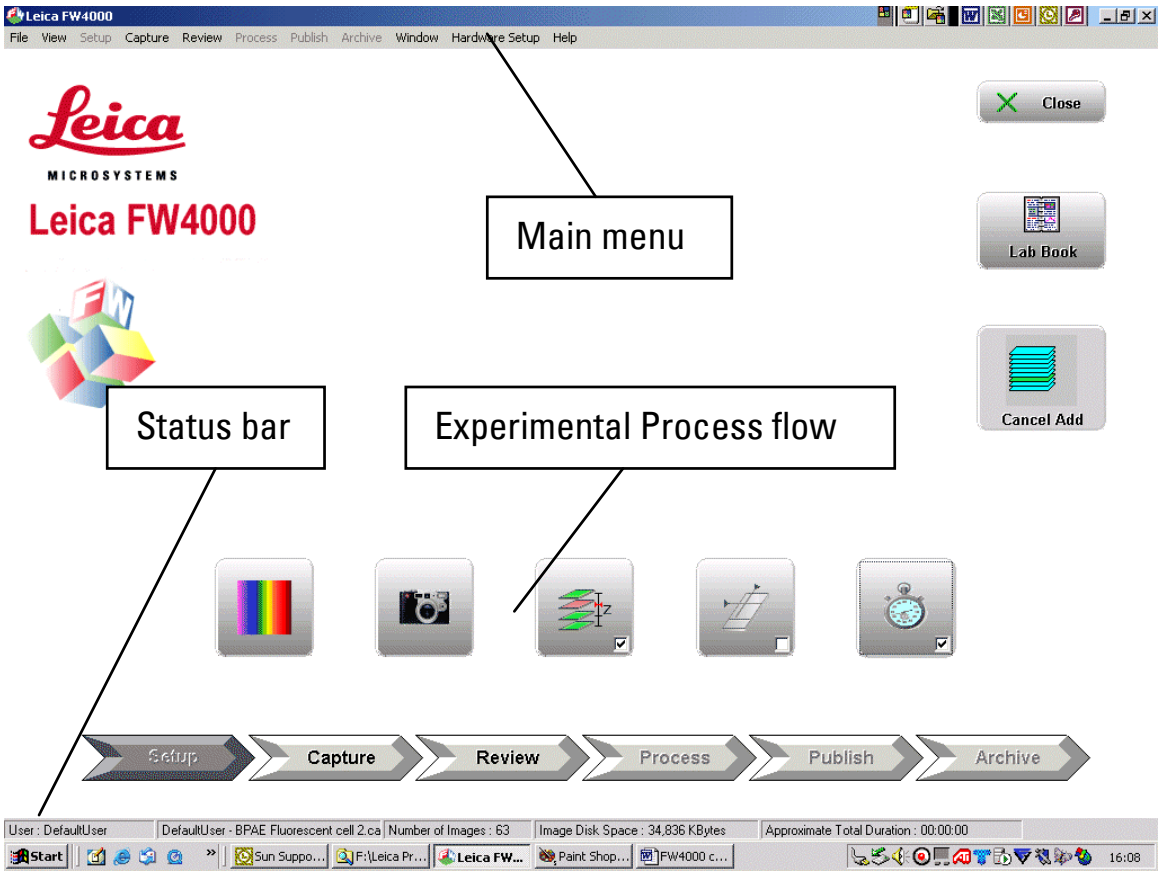
- Introduction to the Leica FW4000 Desktop
- Administration Tools
  - Supervisor's Tools for Managing Users
  - Changing Passwords
  - Changing Users Details
  - User Password Management
- Setting Preferences
  - Setting General Preferences
  - Mapping Keyboard Functions
  - Setting Hardware Preferences
  - Changing the Database Location
- Opening the Lab Book
- Changing the Microscope's Current Configuration
- Viewing Optics Data
- Viewing the Stage Plan
- Setting up the Acquisition Device
- Relative Focus Correction

Introduction to the Leica FW4000 Desktop

After you have logged on, or closed any of the stages in the Experimental Process flow, the Leica Desktop is displayed. You may resize the Leica Desktop to fit your Windows layout. From this Desktop you can perform a number of discrete management actions without needing to enter into the Experimental Process flow. For example, you can:

- Manage the logon (supervisor only).
- Manage users (supervisor only).
- Add new users (supervisor only, the tabs in the dialog are not available when a level 1 user is logged on).
- Log on as a different user.
- Open an existing experiment without logging on again.
- Create a new experiment without logging on again.

Note: Users' ID level is level 1 and the supervisor's is level 2.



➡ Click **Close** to close **Leica FW4000**.

➡ Click **Lab Book** to open the Lab Book.

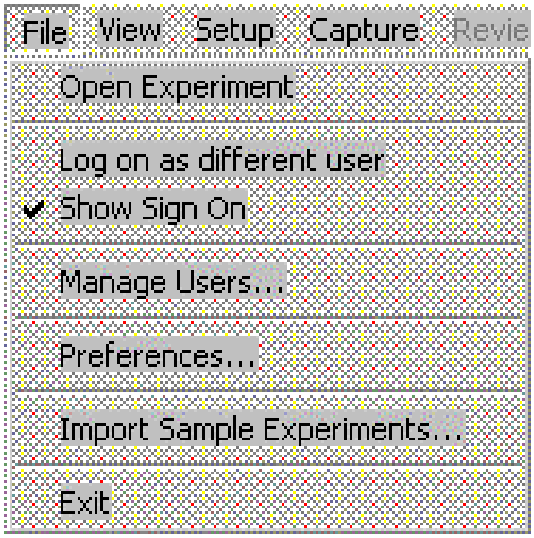
General Administration

- To manage various aspects of system hardware, position the cursor in the centre of the Desktop and right-click the mouse. This action displays the **User Management** menu.

- To display the Desktop properties click **Display Properties**. Toggling the **Show Title Bar** field shows or hides the Desktop title bar.
- To show or hide the status bar at the bottom of the Desktop, click **Show Status**.
- To show or hide the main menu at the top of the Desktop, click **Show Menu**.
- To show the optics data and filter tools, click **Show Microscope**. For a description on using these tools, refer to the section Viewing Optics Data.

Administration Tools

- To show or hide the initial sequence of logon screens, click **FILE** at the main menu and toggle **Show Sign On**.
- To open the Lab Book, click **FILE** at the main menu and then click **Open Experiment,**
- To import a sample experiment, click **File** at the main menu and then click **Import Sample Experiment**. This opens a dialog from which you can choose to open one of 12 sample experiments.

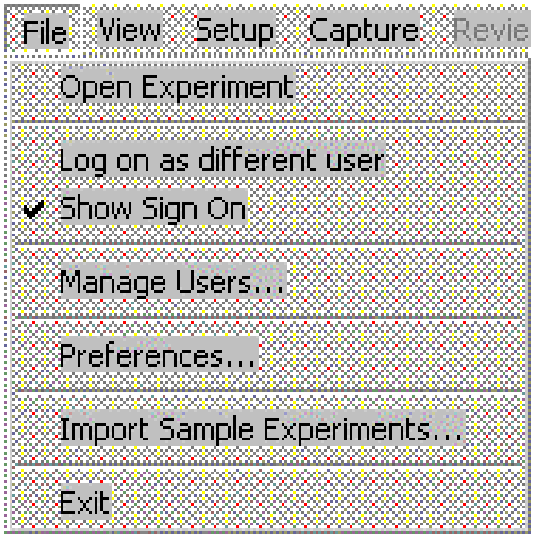


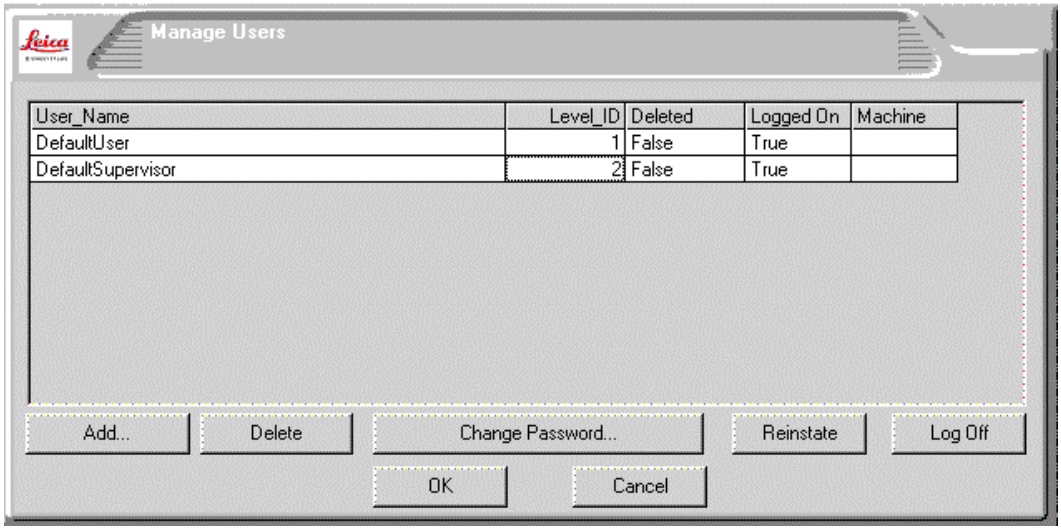
Note: There are 12 sample experiments available that you can use for demonstration purposes.

Note: On initial start-up an Import Sample Experiment dialog appears until you close the dialog permanently. If you wish to use a sample experiment, you can access the dialog through the Import Sample Experiment option.

Supervisor's Tools for Managing Users

To manage users' passwords and ID levels click **FILE** at the main menu and **Manage Users**…. This sequence displays the **Manage User** dialog.





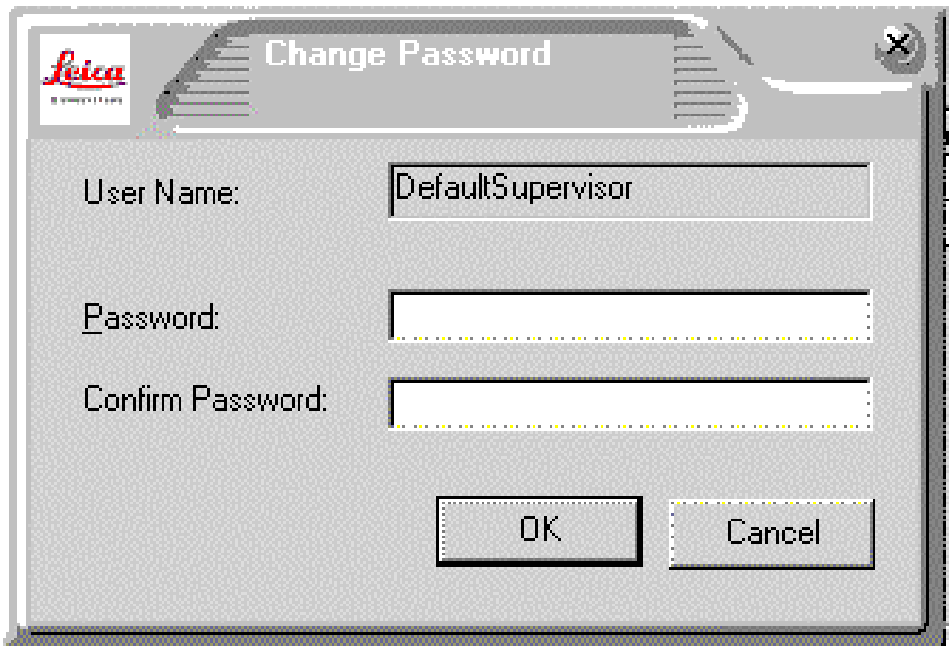
When you are logged on in supervisor view, you may add, delete or reinstate users.

Note: When a user is deleted from the system, only that user or a supervisor can access their experiments. Therefore, either you will have to reinstate that user before you can, for example, archive or delete that experiment or the supervisor will have to do it.

Changing Passwords

To change your password:

1. Click **Change Password** on the **Manage Users** dialog.
2. Type your password into the **Password** field.
3. Confirm your password by typing the identical password into the **Confirm Password** field.
4. Click **OK** to accept the new password, or click **Cancel** to cancel the operation.



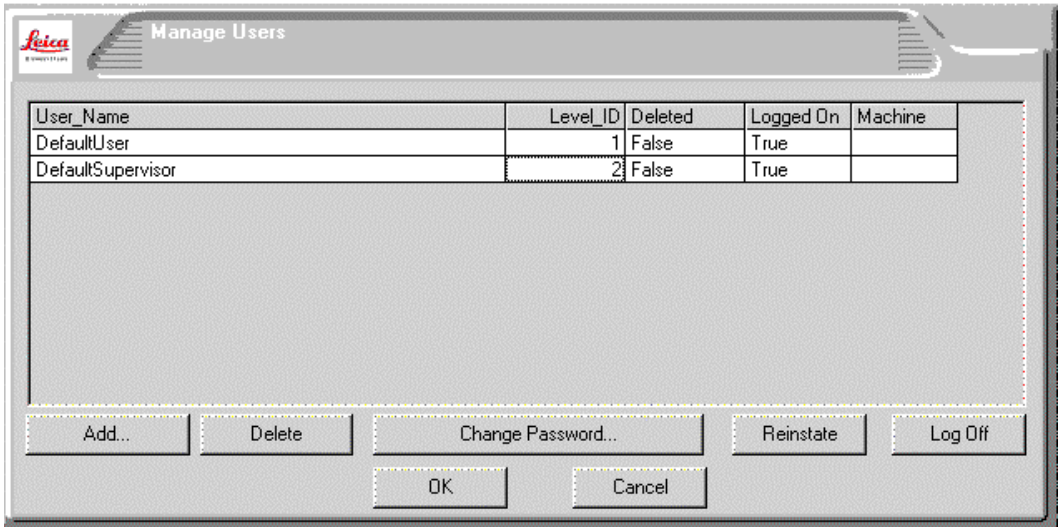
Changing Users Details

The Manage Users dialog allows you to:

- Change a user's level of ID
- Add new users
- Delete existing users
- Reinstate deleted users
- Set a user's status to logged off, particularly when it was erroneously left as logged on because of a previous system failure.

Changing a user's ID level:

1. Click the **Users** tab in the **Edit Users** dialog.



2. Change the Level ID for a user by clicking in the **Level ID** field for the relevant user, and choosing the new ID level from the list of those available.
3. Click **OK** to accept all the changes you have made in the Edit User dialog, or click **Cancel** to cancel the operations.

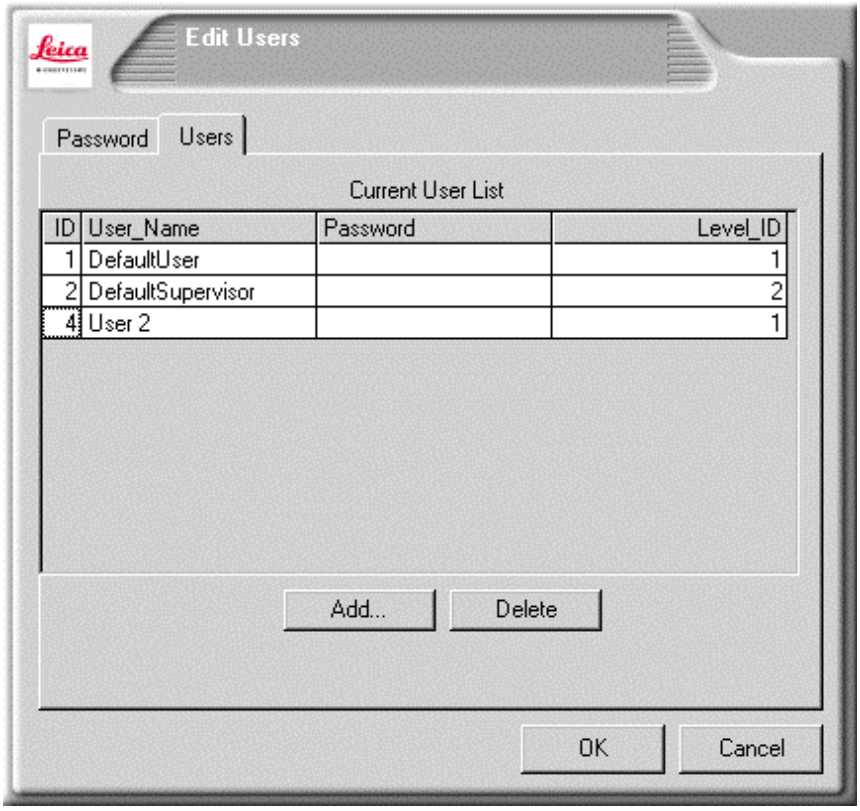
Note: Users' ID level is level 1 and the supervisor's is level 2.

To add a new user:

1. Add a new user by clicking **Add…**.
2. Type the name of the new user into the **New User** dialog field.

3. Click **OK** to add the new user, or click **Cancel** to cancel the operation.
4. Click **OK** to accept all the changes you have made in the Edit

User dialog, or click **Cancel** to cancel the operations.



To delete a user:

1. Highlight the user to be deleted.
2. Click **Delete**.
3. Click **OK** to confirm the deletion, or click **Cancel** to cancel the operation.
4. Click **OK** to accept all the changes you have made in the Edit User dialog, or click **Cancel** to cancel the operations.

Note: The names of deleted users are listed in the Edit Users dialog, but do not appear in the list of users available from the initial logon screen.

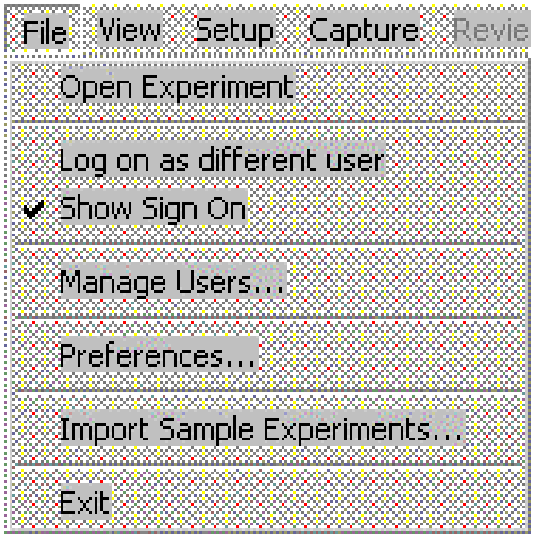
To reinstate a user:

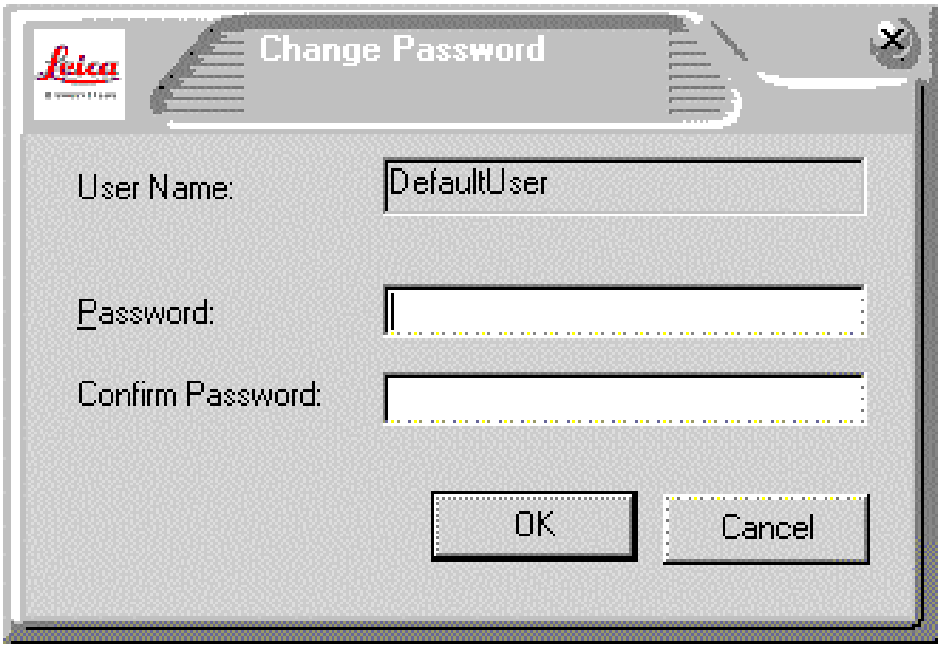
1. Highlight the user to be deleted.
2. Click **Reinstate**.
3. Click **OK** to confirm the reinstatement, or click **Cancel** to cancel the operation.
4. Click **OK** to accept all the changes you have made in the Edit User dialog, or click **Cancel** to cancel the operations.

User Password Management

Users may change their own password as and when required.

➡ To change your password, click **FILE** at the main menu and **Manage Users**…. This sequence displays the **Change Password** dialog.





Note: Only the password details are active.

Only a supervisor has the ability to add new users, as the tabs in the dialog are not available when a level 1 user is logged on.

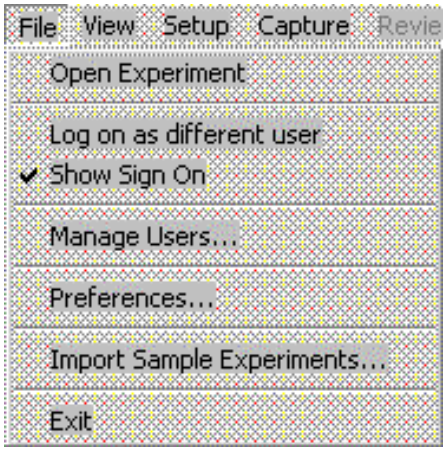
To change your password:

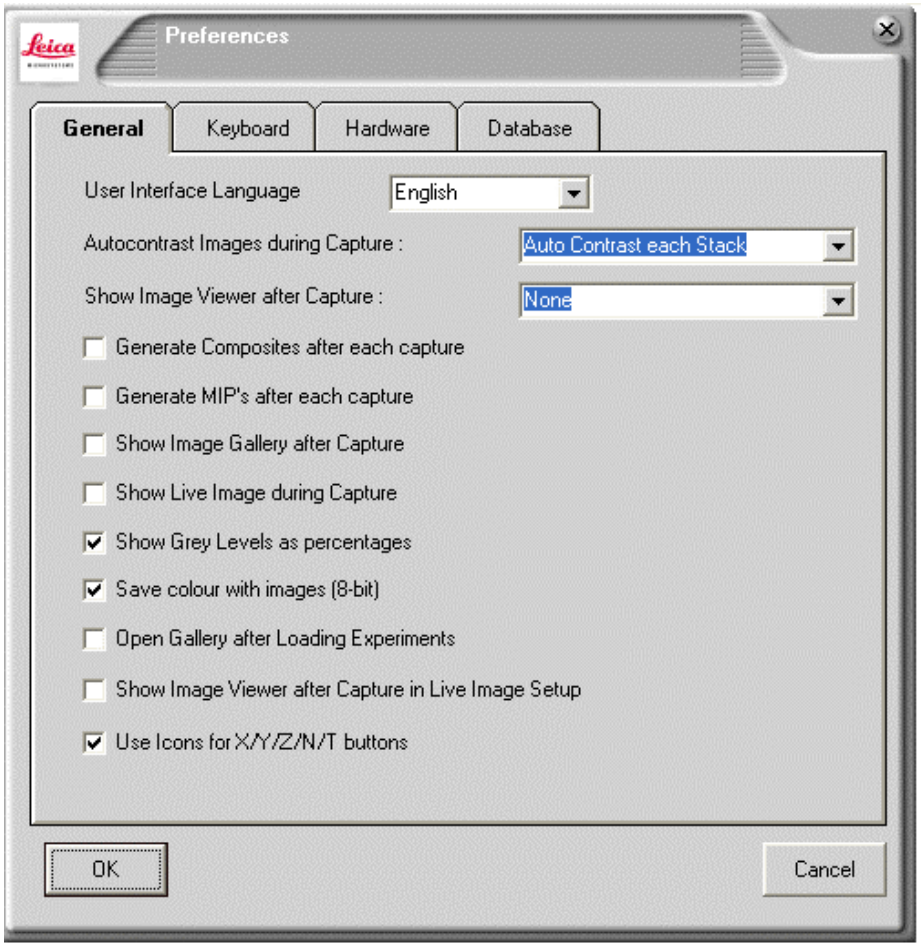
1. Type your password into the **Password** field.
2. Confirm your password by typing the identical password into the **Confirm Password** field.
3. Click **OK** to accept the new password, or click **Cancel** to cancel the operation.

Setting Preferences

The **Preferences** menu allows you to tailor the system and hardware preferences to suit your experimental environment.

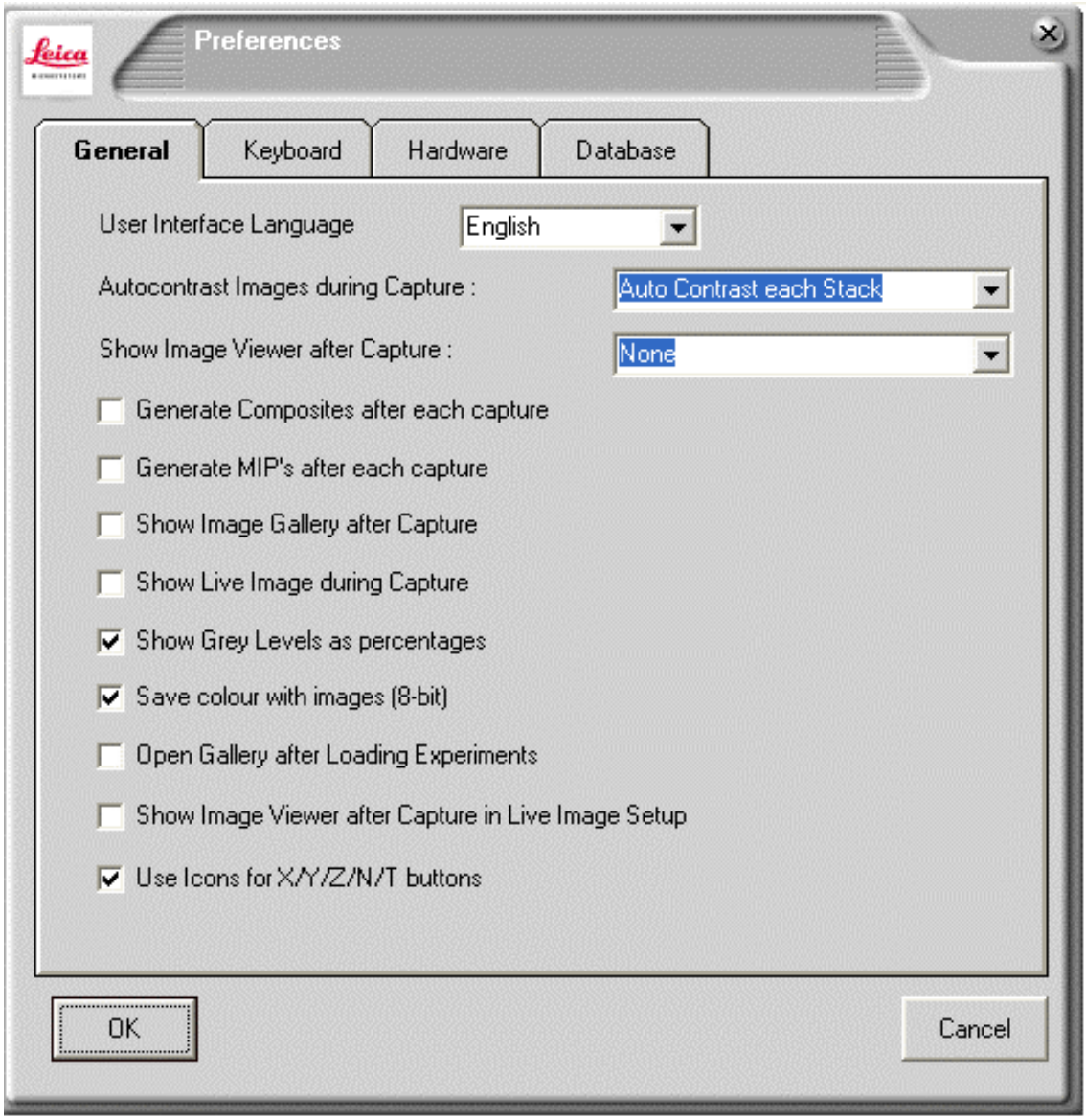
➡ To set system preferences, position the cursor in the centre of the desktop and right-click the mouse, or click **FILE** at the main menu and **Preferences.** This sequence displays the **Preferences** dialog.





Setting General Preferences

➡ Click the **General** tab in the **Preferences** dialog.



➡ Choose the **User Interface Language** from the selection menu.

➡ To generate a composite image, using the probes selected, after each capture, check the **Generate Composites after each capture** box.

➡ To generate the maximum projection of images, check the **Generate MIPs after each capture** box. This generates a maximum projection image from each Z stack and displays it in the gallery.

➡ To display an image after it is captured, and the first and last image refers to first in a time/z stack sequence (and so on), check the **Show Image Viewer after Capture** box with the 'none, first, centre or last' option selected.

Note: If you have selected one of **first**, **centre**, or **last** from the **Show Image Viewer after Capture** menu, then the image gallery display is overlaid with the image viewer.

Tip: To automatically display only the image gallery, set the **Show Image Viewer after Capture** menu to none.

➡ To automatically show the image viewer after taking a single camera image, check the **Show Image After Capture in live image setup** box.

Note: This allows you to continue capturing images whist viewing the images that have already been captured alongside in the image viewer.

➡ Check **Show Grey levels as percentages** to show the grey level value as a percentage rather than an integer in the range 0 to 255.

➡ To map the grey levels in an image from the camera to the 256 grey levels that are displayed, select one of the options from the **Auto Contrast Images during Capture dropdown list**. The options are:

- Use the contrast values defined for each probe
- Auto contrast each image separately
- Auto contrast an entire stack, using the same contrast values for each image in the stack

➡ To display the live image, allowing you to monitor the image during the experiment, check the Show live image during capture box.

Note: Auto contrast is used only on high-resolution cameras that have more than 256 possible grey levels.

➡ To save the colours assigned to the image by the software when a monochrome camera is capturing the image, check the **Save Colour**

During Capture box.

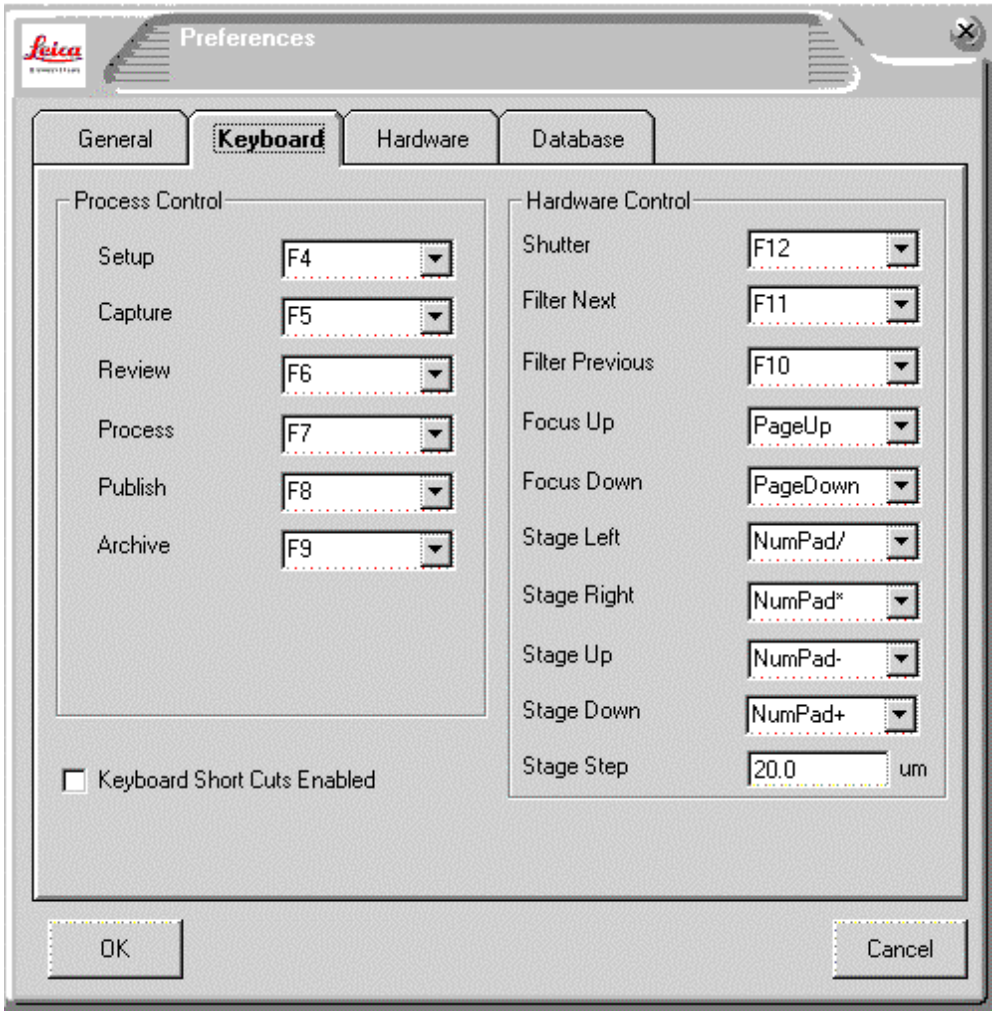
➡ To autoload and launch the Image Gallery when you open an experiment, check the **Open Gallery During Capture** box.

Mapping Keyboard Functions

The Keyboard tab allows you to map the keyboard function keys to the six steps in the Experimental Process flow.

To map the keyboard functions:

1. Click the **Keyboard** tab in the **Preferences** dialog.



2. Click the function key menu for Setup.
3. Highlight the function key you wish to use to represent Setup.
4. Repeat steps 2 and 3 for each of the Process and Hardware

Control functions.

5. Click **OK**, or click **Cancel** to cancel the mapping.

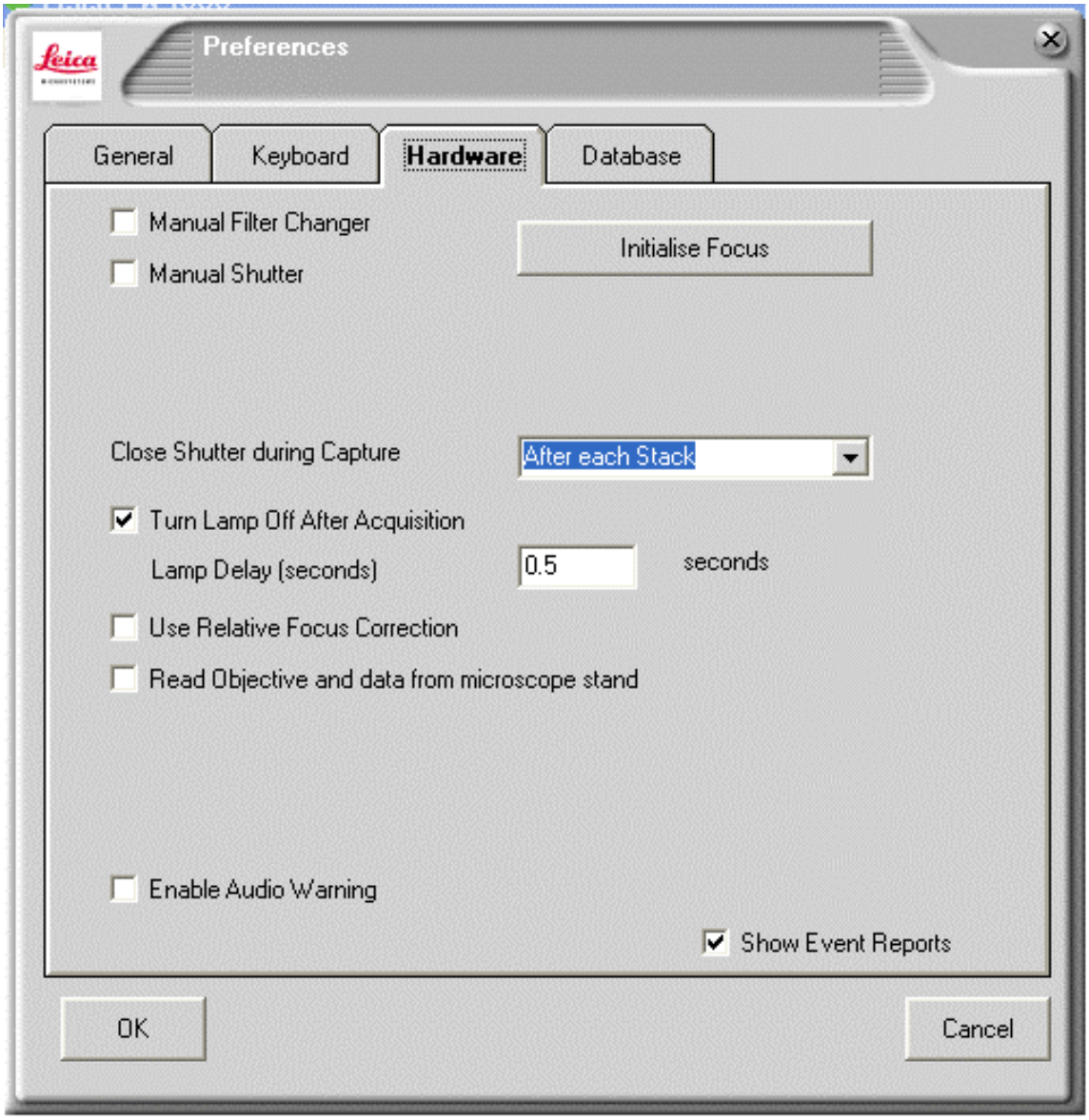
Note: You can also set the distance the stage moves during stage left, right, and so on, movements in the Stage Step box.

WARNING: Do not use NUMPAD keys when entering numerical information for controlling hardware, for example, when adjusting the focus position, as this causes your stage to move unexpectedly.

Setting Hardware Preferences

The Hardware tab allows you to set up the hardware for manual and automatic microscopes.

➡ Click the **Hardware** tab in the **Preferences** dialog.



➡ To change a filter with a manual microscope, check the **Manual Filter Changer** box.

Note: When the Manual Filter Change box is checked, you will be prompted to ensure the correct filter is in position.

➡ To open the shutter and ensure that light is reaching the sample, check the **Manual Shutter** box.

➡ To initialise the focus when your microscope has an automated X/Y stage, click the **Initialise Focus** button or external control device.

➡ **Closing the shutter** gives you options on when you want the camera shutter to close. The options available are:

- Only at the end of capture
- After each stack
- After each sequence
- After each image

➡ To turn the lamp off after acquiring your images, check the **Turn Lamp off after acquisition** box.

Tip: You should check the **Turn Lamp off after acquisition** box when there is a long time delay between capturing images or sequences of images. Also, in these cases you might want to specify lamp delay. When you have defined a short time sequence, leave this box unchecked.

➡ To allow sufficient time for the lamp to be at its optimum brightness, set an appropriate **Lamp Delay** (measured in seconds). This is important after you switch on the lamp during a long time sequence, when capturing brightfield images.

➡ To cause a 'bleep' to be sounded when a new dialog appears or other event completes, check the **Enable audio warning** box. Unchecking the box turns off the 'bleep' sound.

➡ To specify that the microscope details are to be read from the microscope rather than entered manually by the user when creating a new experiment, check the **Read objective and data from microscope stand** check box.

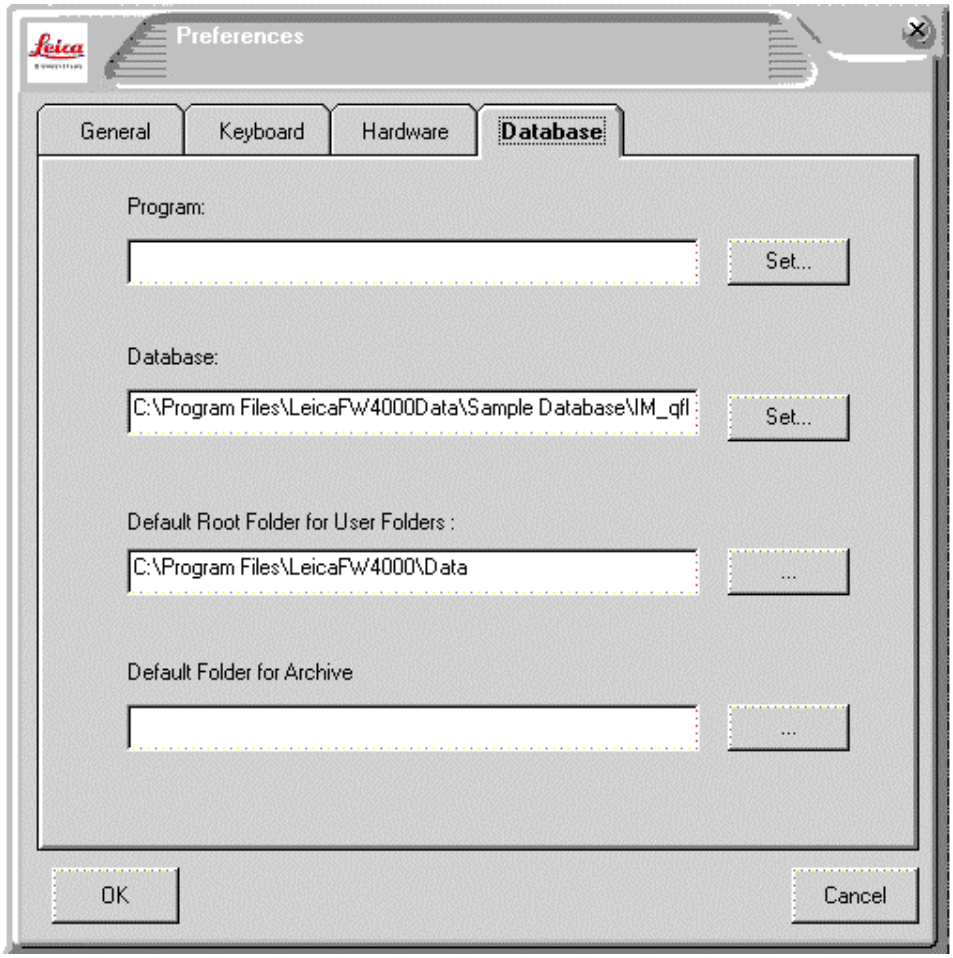
Refer to: The

Relative Focus Correction description on page 49 of this manual for a description on correcting the relative focus.

Changing the Database Location

The Database tab allows you to set the paths for the location of the **IM1000** database.

➡ Click the **Database** tab in the **Preferences** dialog.



➡ Change the program, if required, by clicking the Program **Set** button. This displays the **Set Database Program** dialog where you can browse and select a new location. You can also type the location of the database directly into the text box. This allows you to point **IM1000** to a database file in a different location, and/or to a different database. This feature is useful if you are accessing the database over a network, or if you have created your own database. The new database must already exist in the named location.

- Click on the **Database Set** button to change the location of the **IM1000** database. This displays the **Set Database Program** dialog where you can browse and select a new location. The current location is displayed in the text box alongside the button. You can also change the location by typing the new location into the textbox.

- Click on the **Default Root Folder** to change the location of the **IM1000** default root folder. This displays the **Set Root Folder Location** dialog where you can browse and select a new location. The current location is displayed in the text box alongside the button. You can also change the location by typing the new location into the textbox

- Click on the **Default folder for archive** to change the location of the **Leica FW4000** default archive folder. This displays the **Set Archive Location** dialog where you can browse and select a new location. The current location is displayed in the text box alongside the button. You can also change the location by typing the new location into the text box.

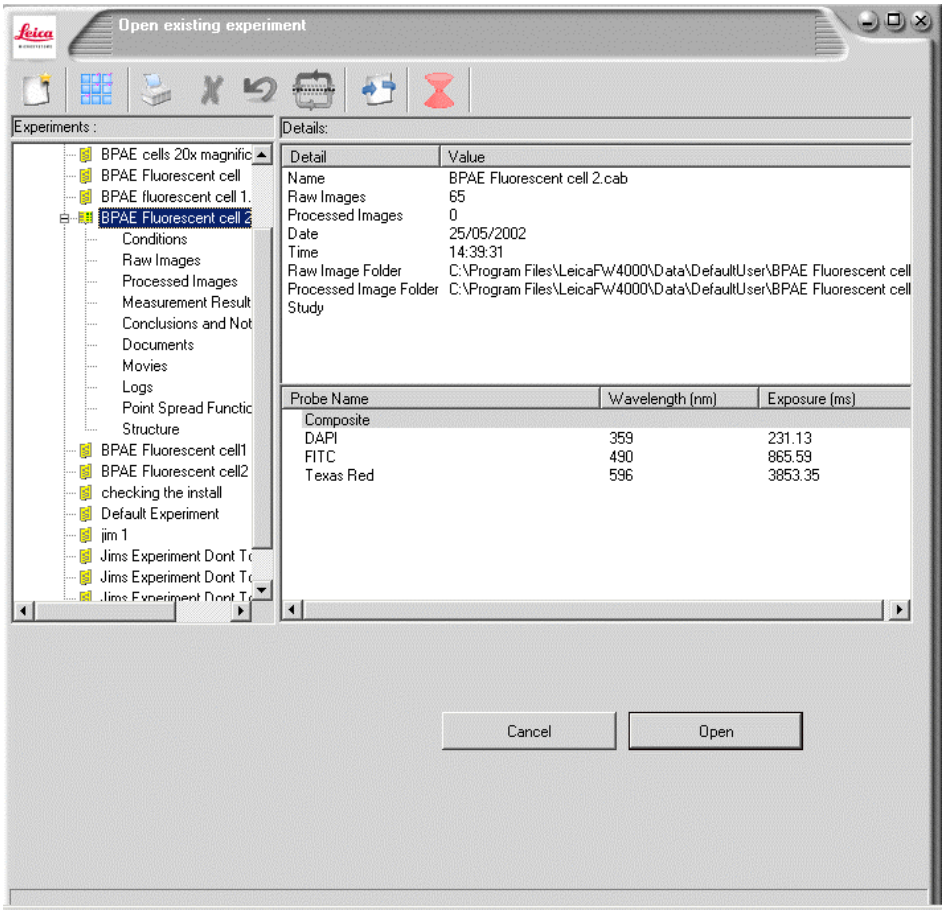
Opening the Lab Book

The Lab Book is the central controlling element for choosing the experiment to work in. Also, it is the interface to the **Leica FW4000** database.

The structure of the Lab Book for each experiment is identical, and is set up for you by default. It contains your data, captured images and results for each of your experiments. The **Open Existing Experiment** dialog shows the structure of a Lab Book.

You can reach the Lab Book from the **Leica FW4000** Desktop without having to restart **Leica FW4000**.

➡ To open the laboratory book, click **Lab Book**. The **Open Existing Experiment** dialog is displayed.

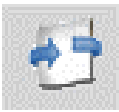


➡ To create a new experiment, click the **Start New Experiment** button.

Refer to: *Leica FW4000 Quick Start Guide* for further information on the procedure for creating a new experiment.

➡ You can view the image gallery of the experiment currently selected in the Lab Book from **the Create a New Experiment** or **Open an Existing Experiment** dialogs. To do this, click the **Image Gallery** button.

➡ When you have expanded the Lab Books for your experiments you can easily collapse all the hierarchies by clicking the **Redisplay Data** button.



Note: When you create a new experiment in the Lab Book, an image set called 'Processed Images' is created automatically in the experiment. This is the default image set used when the **Saving Images** dialog appears. There are a number of places where this dialog is used after clicking **Apply** or **Apply to selected** when using the deconvolution or image enhancement tools.

Once you choose where the processed images should go, the image set that is currently 'in session' changes in the drop-down list at the bottom left of the image viewer. This provides a mechanism for you to switch image sets from the **Image Viewer**. The new image sets are also available in the Image Gallery and in the tree view in the Publish Results dialog.

Refer to: *Chapter 7, Using a Processed Image Set* for further information.

To delete an experiment:

1. Select **Users** or a particular user in the tree view.
2. Select an experiment in the right hand view.
3. Click the **Delete** icon

Note: Ordinary users can only delete their own experiments.

To delete an image from the Lab Book:

1. Click the **Raw Image or Processed Image** radio button, which

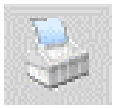
displays the Tree View node.

2. Highlight the images to be deleted.
3. Click the **Delete** icon.
4. Click **OK** in the **Confirm Deletion** Dialog box.

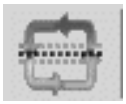
➡ To undo a delete action, click the **Undo** icon.

Note: You can undo only the most recent Delete Experiments action, that is, the ones deleted the last time you pressed the Delete icon.

➡ To print the details of the experiment selected in the tree view, click the **Print** icon.



➡ Click the **Properties** icon to change which details of a user's experiments are shown in the right hand panel when a user is selected in the tree view. Also, you can filter the experiments that are shown.



Refer to: *Chapter 12, Archiving Experiments* for further information.

Note: You can search for all experiment performed on a certain day, or search for experiments that have z-stacks.

To access some extra functions, right click on the experiment name to bring up the menu options:

- Unlock experiment

  Use this to unlock an experiment when the database has 'locked' it as a result of a problem. You rarely need to use this option
- Unlock all experiments

  Use this to unlock all experiments when the database has 'locked' them as a result of a problem
- Allow deletion

This option is checked by default. Uncheck it if you want to prevent your experiment being deleted

- Reset gallery options

  Use this to reset any image selection options that have been applied in the gallery by the Select required images option

- Allow changes

  This option is checked by default. Uncheck it if you want to prevent experiments being changed in any way

- Display experiment details

  This option is only available when you click Conditions, and displays information about the status of the experiment

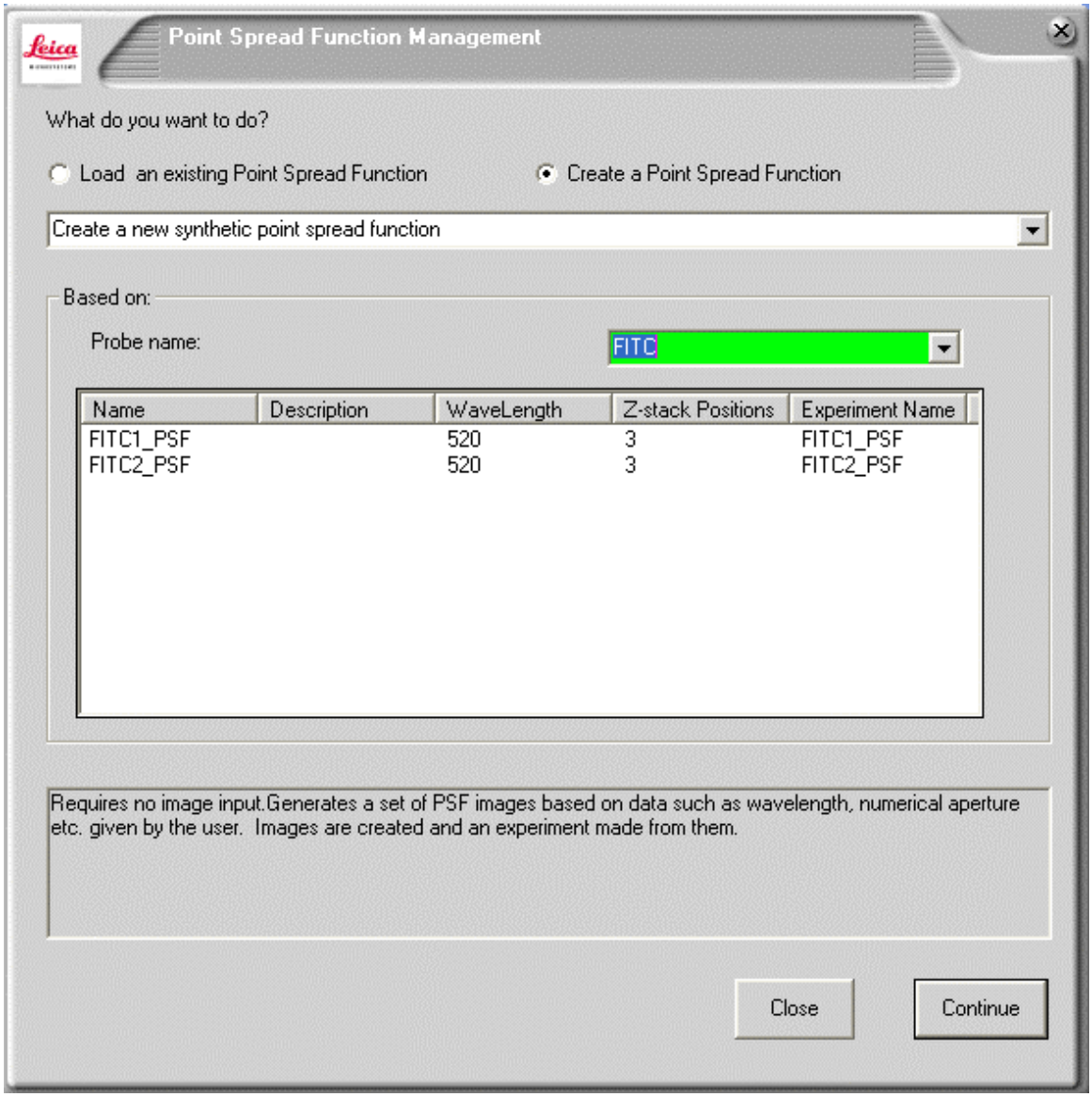
Note: When Allow changes and Allow deletion are unchecked, the icons on the experiment change to a no entry or a red key symbol.

Point Spread Function Management

Leica FW4000 applications have facilities for creating and managing point spread functions for use in deconvolving captured images with **Leica Deblur and 3D Visualise**. You can use a captured experiment, such as an image of a bead, as a Point Spread Function (PSF). You can attach a PSF to an experiment for use later to improve the captured images.

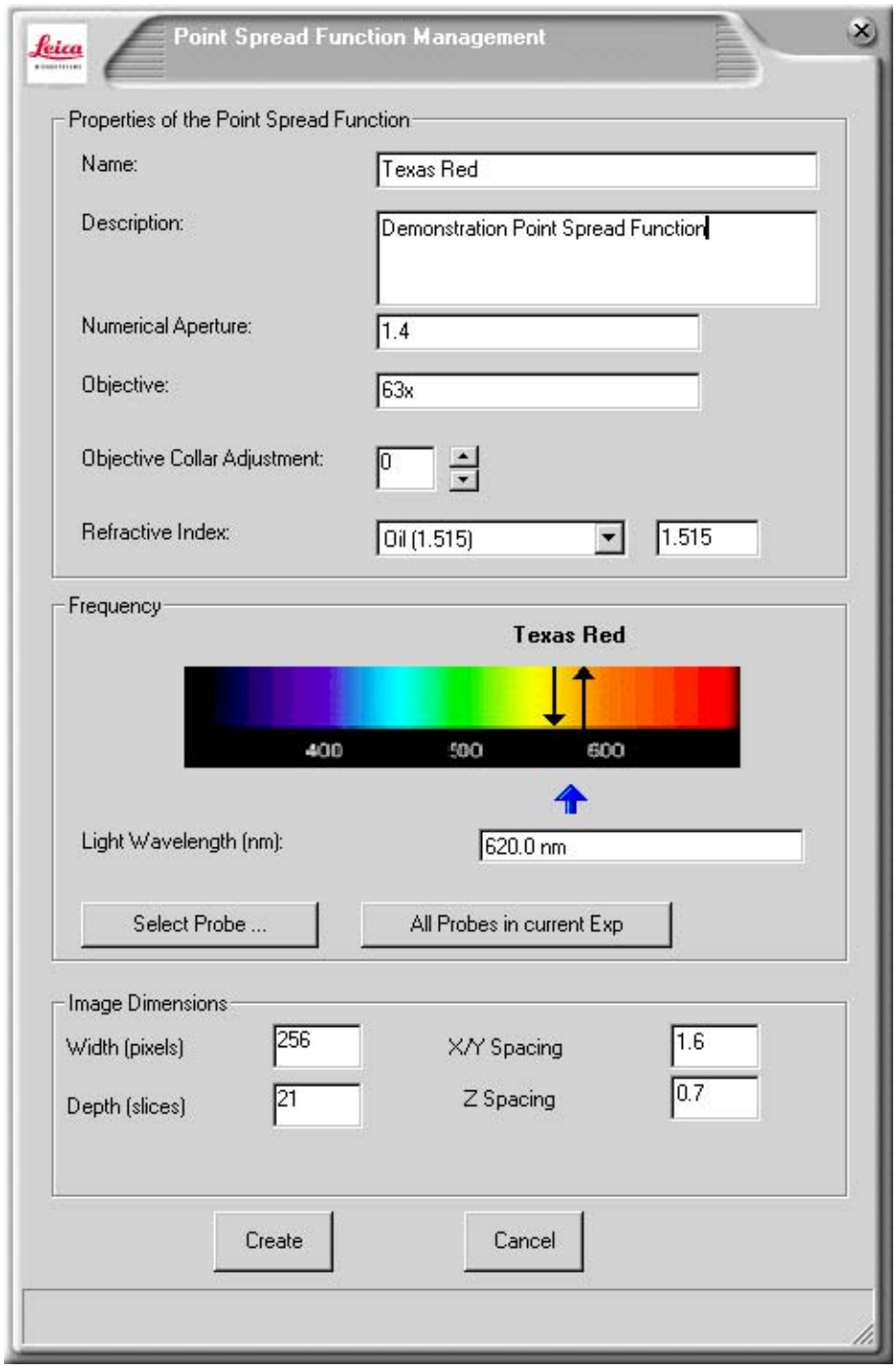
To implement Point Spread Function management:

1. Click the **Point Spread Function Management** button. This action displays the **Point Spread Function Management** dialog.



Note: The description of the PSF function selected is displayed in the dialog when you have selected your activity.

2. Choose the task you wish to perform.
3. Choose the probe you wish to use.
4. Click **Continue** to display the dialog shown below, or click **Cancel** to cancel the operation.



➡ To define a new synthetic PSF, type the physical parameters that define the image that will be computed into the **Properties of the Point Spread Function** area.

➡ To capture the microscope features, type values for the Numerical Aperture of the microscope objective, the Refractive Index of the

immersion fluid, and any information that you will find useful into the **Properties of the Point Spread Function** area.

➡ Type the wavelength of light for the probes that are set for the current experiment, into the **Frequency** area of the dialog. You can take the wavelength value from the probes for the current experiment or from the list of available Fluorochromes.

Note: You must specify the X/Y and Z numerical fields in Microns.

Note: The PSF will be different for different wavelengths.

➡ Type the dimensions of the physical image size into the **Image Dimensions** area of the dialog. The number of planes is given. The distance in microns between adjacent pixels is needed, as is the separation in microns between image planes.

Note: Each plane of the image stack will be square, with its width in pixels specified.

When you have defined all of these parameters, the software creates a new PSF to simulate the appearance of a fine bead viewed under those conditions.

➡ To allow the PSF to mimic the behaviour of the Correction Collar, set the **Objective Collar Adjustment** to an appropriate value.

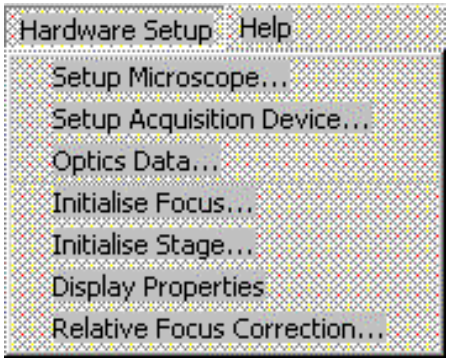
Note: This reshapes the PSF to obtain better levels of detail from the image. Therefore, it is possible to produce related PSFs (by varying this setting) that can each be used in succession with a difficult image to extract the most information from it.

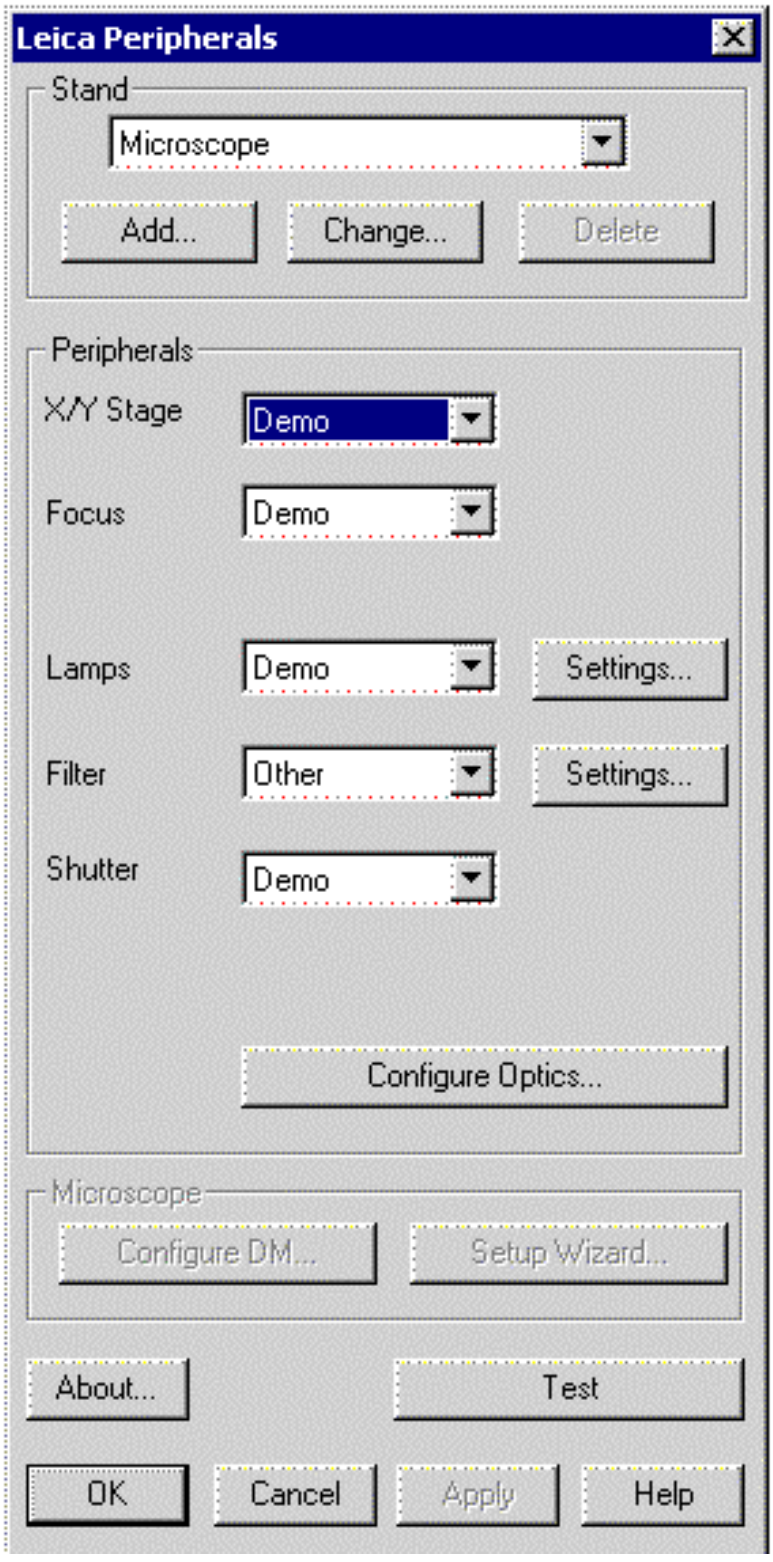
➡ The PSF images will be stored in a new experiment. To define the location of this experiment, select the browse button marked …
and browse to the desired location.

Changing the Microscope's Current Configuration

You can change the current microscope configuration details. It will already be set up to match your hardware and you will not normally be required to change these settings. The dialog may be used to verify that the microscope configuration is correct, test the connection between the computer and the microscope, or alter the software configuration if the microscope options have been changed.

➡ To change the microscope configuration, select **Hardware Setup** from the main menu and click **Setup Microscope**. This displays the Setup Microscope dialog.



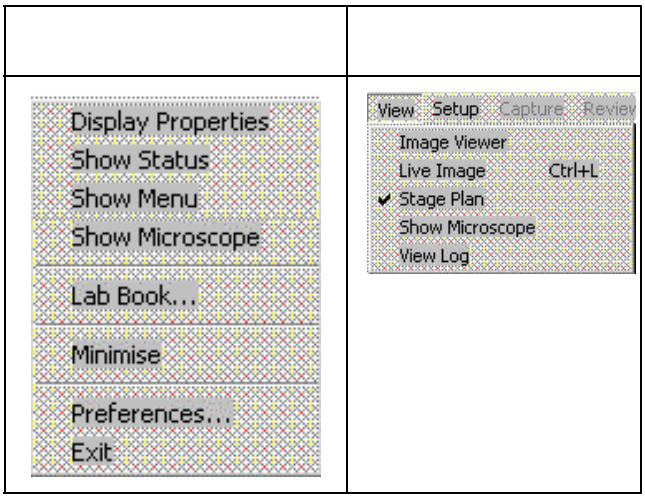


Refer to: the *Leica Server Documentation* for more information on this dialog.

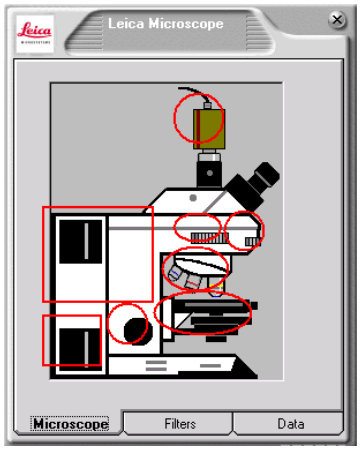
Viewing Optics Data

To view the optics data:

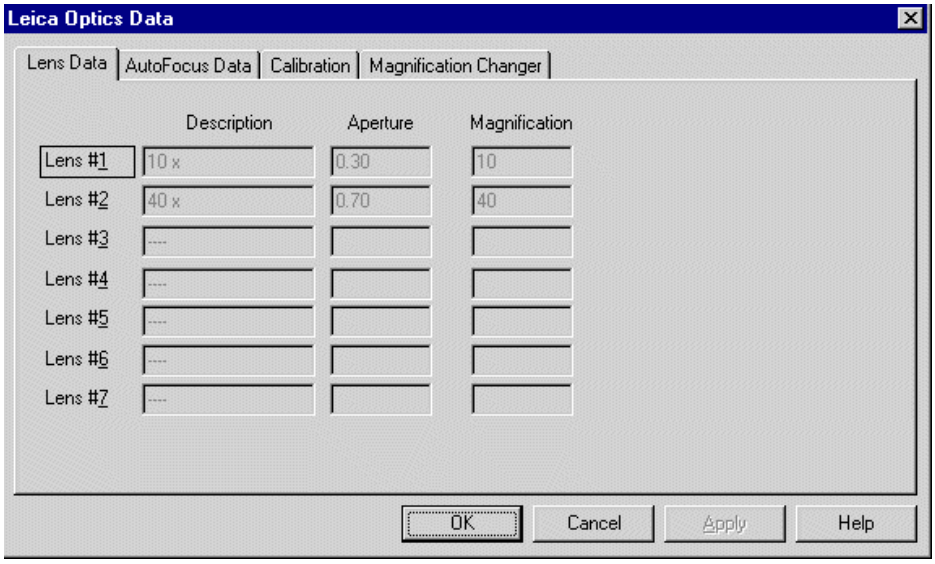
1. Access the menu by performing one of:
   - Right click to display the drop-down menu, or
   - Open the **View** menu from the main toolbar
2. Select **Show Microscope** from the displayed menu and click **Data**.



➡ Double click on the Objectives shown on the screen to see the Leica Objective dialog. You may change a particular attribute of the microscope from the new screen displayed.



➡ To display Leica Optics Data, click **Optics Data** from the **Hardware Setup** menu in the main toolbar.

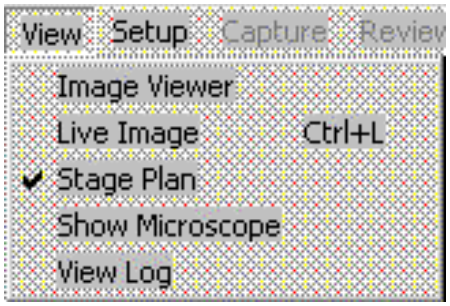


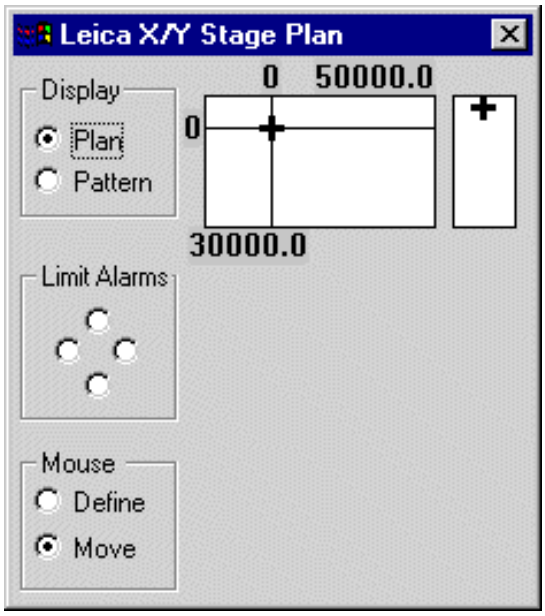
Refer to: the *Leica Server Documentation* for more information on this dialog.

➡ To view the optics filter, right click to display the drop-down menu, or open the VIEW menu from the main toolbar, select **Show Microscope** from the displayed menu and click **Filter.**

Viewing the Stage Plan

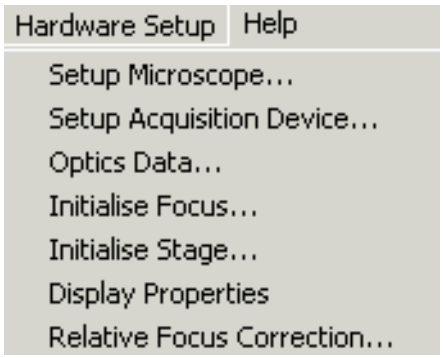
➡ To view the stage plan, select **View** from the main menu and click **Stage Plan**. This displays the **Leica X/Y Stage Plan** dialog.



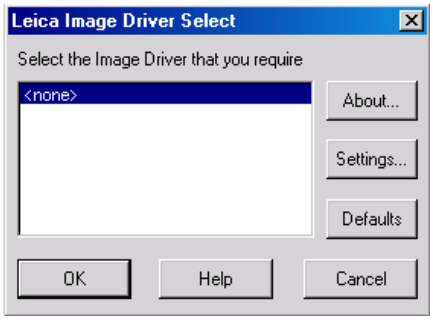


Setting up the Acquisition Device

To change the camera you are connected to, select **Hardware Setup** from the main menu and click **Setup Acquisition Device** dialog.



This displays the **Setup Acquisition Device** dialog.



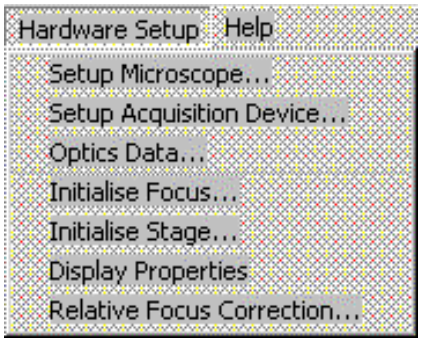
Refer to: *Chapter 4, Defining Live Image Acquisition* for more information.

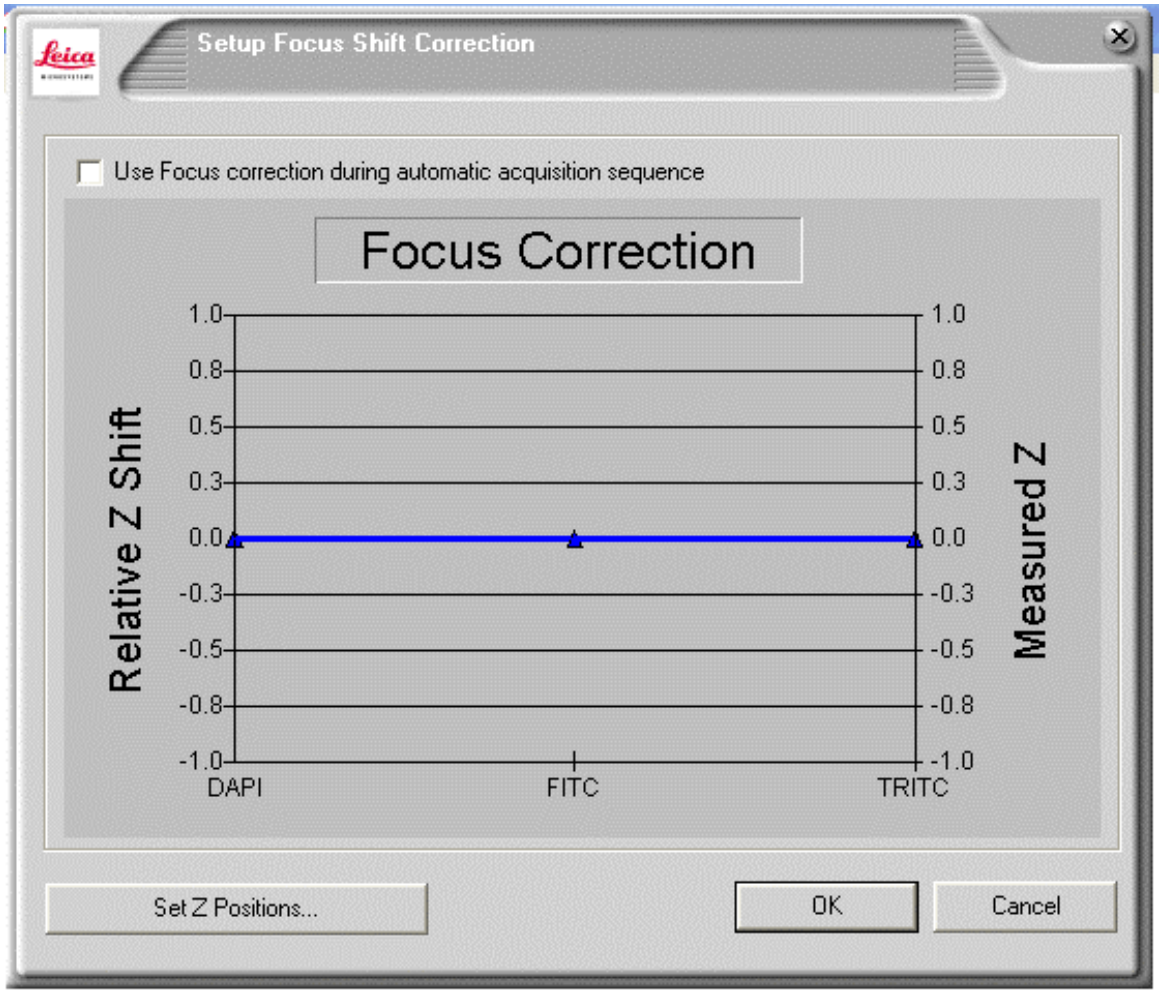
Relative Focus Correction

You can measure and correct the Z-axis shift between filter cubes. By using the **Setup Focus Shift Correction** dialog, you can compensate for any relative focus shifts between filters during the automatic acquisition of component images.

To enable focus correction:

1. Click **Hardware Setup** in the main menu and click **Relative Focus Correction**. This displays the **Setup Focus Shift Correction** dialog.





2. Click **Set Z Positions** displays the **X/Y** dialog.
3. Adjust the microscope until the image is in focus.
4. Repeat this process for each probe.
   These values are then stored and can be applied if desired by accessing the Preference section on the Hardware tab. Use Relative Focus Correction either by:
   - Selecting the option from here
   - Switching it on by checking the box under the Settings menu on the Live image screen

Chapter Three: Quick Start into Experimental Setup

Chapter Overview

This chapter contains the following topics:

- Introduction
- Lab Book
- Experimental Process
  - Setting up an Experiment
  - Capturing Images
  - Reviewing Images
  - Processing Images
  - Publishing Results
  - Archiving Experiments

Introduction

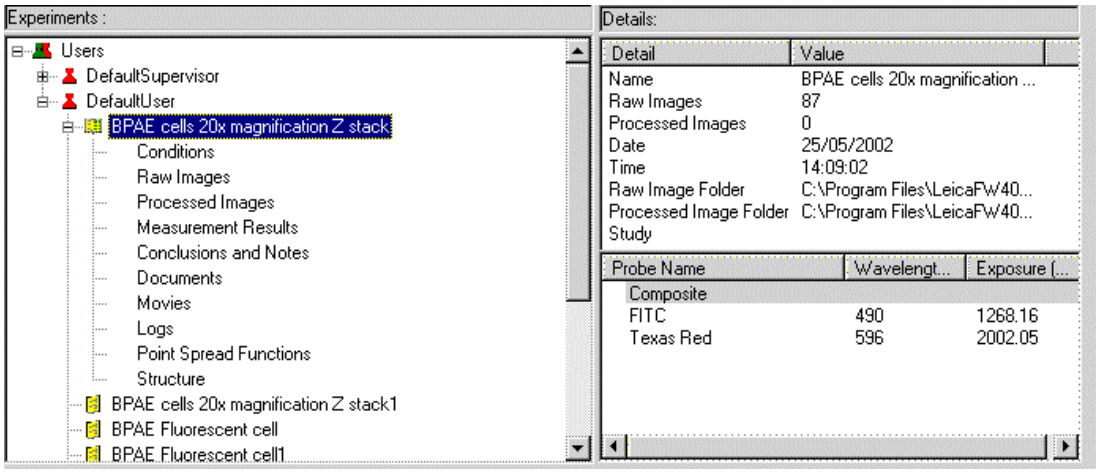
Leica FW4000 has an easy-to-use and intuitive interface. The environment provided allows you to perform experiments and tasks quickly and easily.

Tip: When you have read through this chapter and *Leica FW4000 Quick Start Guide*, create a test experiment and familiarise yourself with the **Leica FW4000** software.

Lab Book

The Lab Book is the central controlling element for choosing the experiment to work in. It is also the interface to the **Leica FW4000** database.

The Lab Book structure is set up by default for your data, captured images and results. The electronic files are structured very much like a laboratory workbook and contain the following areas or topics for your data:



Refer to: *Chapter 2, Opening the Lab Book* for more information on using the full set of facilities available with the Lab Book.

Experimental Process

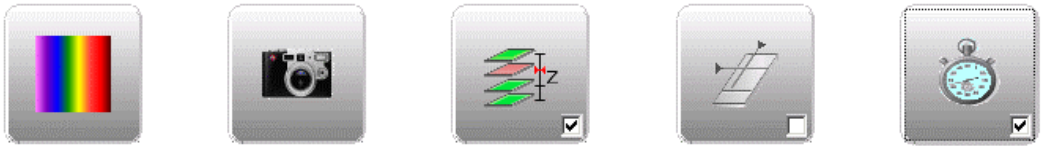
This chapter gives you an overview of the six major elements in the Experimental Setup that you will use when performing an experiment.

When you have logged on and chosen an existing experiment or created a new experiment, the **Leica FW4000** software displays the six-stage Experimental Process Buttons. You use these buttons to move through an experiment lifecycle.

You can pause at any stage in the process. **Leica FW4000** automatically saves the data setup and captured images in the laboratory workbook for your experiment. To continue with the experiment, open the paused experiment when you next log in, and continue from the appropriate stage in the process flow.

Setting up an Experiment

Clicking **Setup** displays controls for the five physical components you can set up. The check box, where applicable, must be checked before you can set up that aspect of the experiment.



The XY dialog opens automatically when you click **Setup**. This allows you to change camera and microscope settings to create optimal images.

You can change the order of the five physical component icons by right clicking on an icon, and then dragging and dropping it in the desired position in the sequence. In previous versions of the software, these buttons contained text. This classic look is available as an option in the preferences dialog.

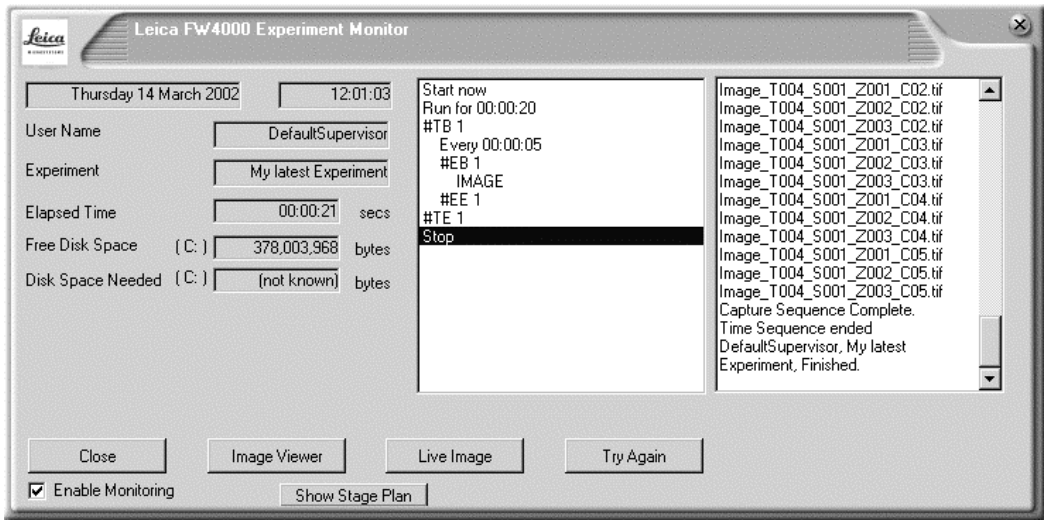
In order the above buttons allow you to define:

- Fluorochromes selected from the fluorochrome database, and define the settings to be used for each fluorochrome selected Users may also add their own customised fluorochromes
- Size and position of the image on the camera's chip
- Z-stack and choose the spacing between the acquired images
- Discrete positions on the sample where you require images to be captured
- Time sequencing routines used to run the experiment

Refer to: *Chapter 4: Setting up an Environment for Experiments*.

Capturing Images

Clicking **Capture** in the experimental setup causes the image capture sequence you set up earlier to run automatically. You may stop the sequence at any time and view the images taken during the sequence run. Clicking **Try Again** runs through the sequence again from the start.



Note: This way of working provides a hands off, automated image capture sequence (such as time sequence or Z-stack image sequences) method.

Refer to: *Chapter 5: Capturing Images.*

Alternatively, set the image frame and exposure you require, whilst the image is **Live**, and click the **Camera** button. Click the **Image Viewer** button to display the Image Viewer where you will see the captured image displayed. Adjust the size and screen position of the Image Viewer so the image viewer and XY dialog are open alongside each other.

Note: This way of working is recommended during optimisation of the image acquisition parameters.

Reviewing Images

The **Image Gallery** displays thumbnails of the component and composite images, which you can select and magnify. The options supplied with the gallery allow you to:

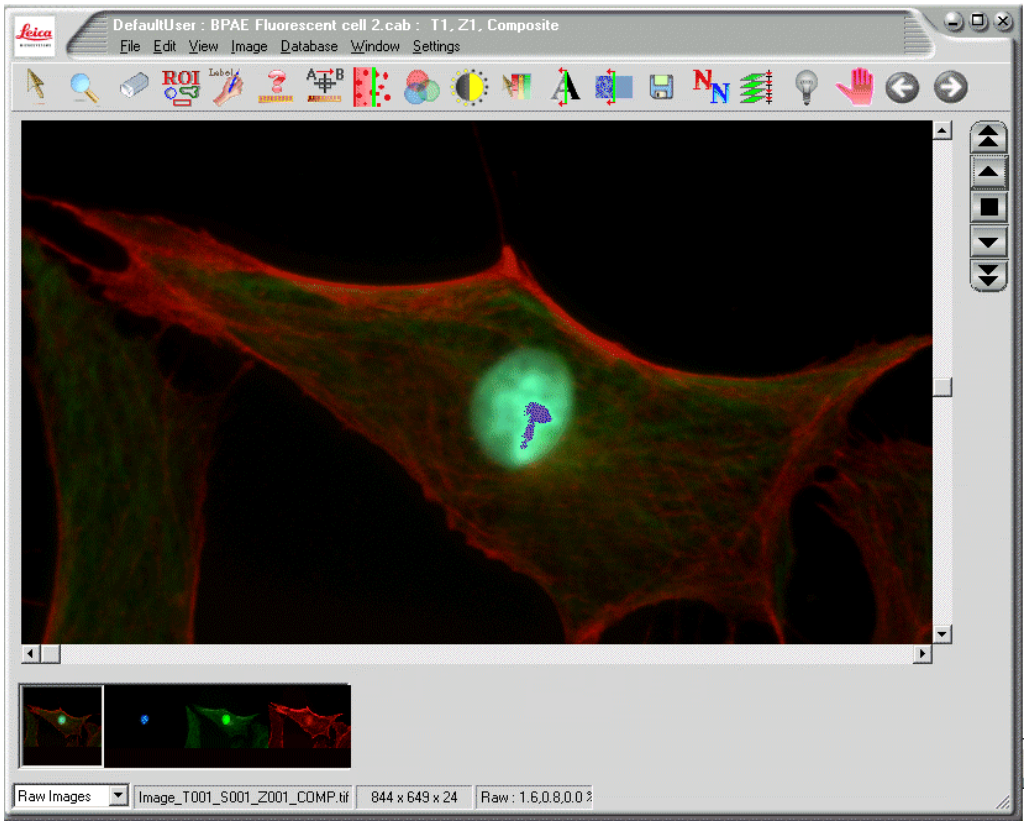
The gallery is an optional component.

- Combine images.
- Change the order in which the images are displayed by probe, fluorochrome, time point acquired and Z position.
- Select and deselect images for inclusion in processing or publishing activities.
- Redefine elements of the experiment.
- Recall experimental details.
- Use filtering options.
- Launch an image viewer.

Refer to: *Chapter 6, Reviewing Images*.

Processing Images

The **Image Viewer** is a full display of the component and composite images, which you have selected.


DefaultUser : BPAE Fluorescent cell 2.cab : T1, Z1, Composite
File Edit View Image Database Window Settings
ROI
Raw Images
Image_T001_S001_Z001_COMP.tif
844 x 649 x 24
Raw : 1.6,0.8,0.0

The options supplied with the viewer allow you to process images by:

- Defining and selecting regions of interest.
- Performing measurements on your experiment.
- Mixing the channels to enhance your images.
- Rescaling your images.
- Enhancing images by removing background noise, smoothing, sharpening, correcting misalignment and converting images to brightfield.
- Performing no neighbours and nearest neighbours deconvolution.

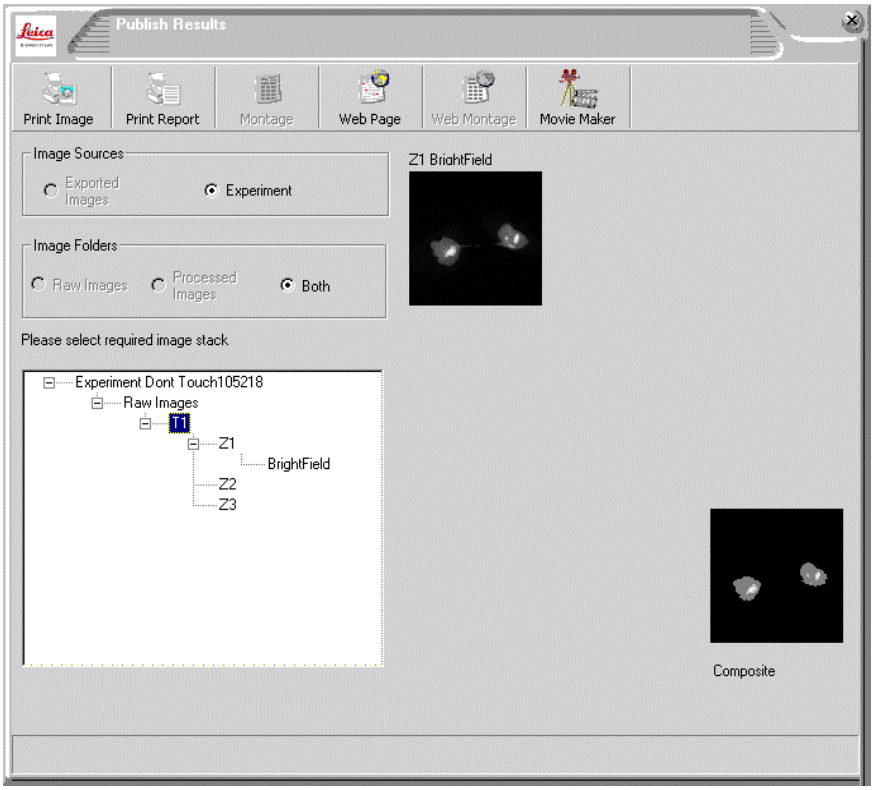
Refer to the following chapters for information on how to use the image processing tools:

- *Chapter 7, Processing Images and Deconvolution*
- *Chapter 8, Performing Measurements*
- *Chapter 9, Enhancing your Image*
- *Chapter 10, Advanced Deconvolution*

Publishing Results

The methods available for publishing results via the **Publishing Results** dialog are:

- Print a selection of images.
- Print a report with images embedded.
- Montage images for Z-stack or time lapse experiments.
- Make .avi movie files from collections of images.

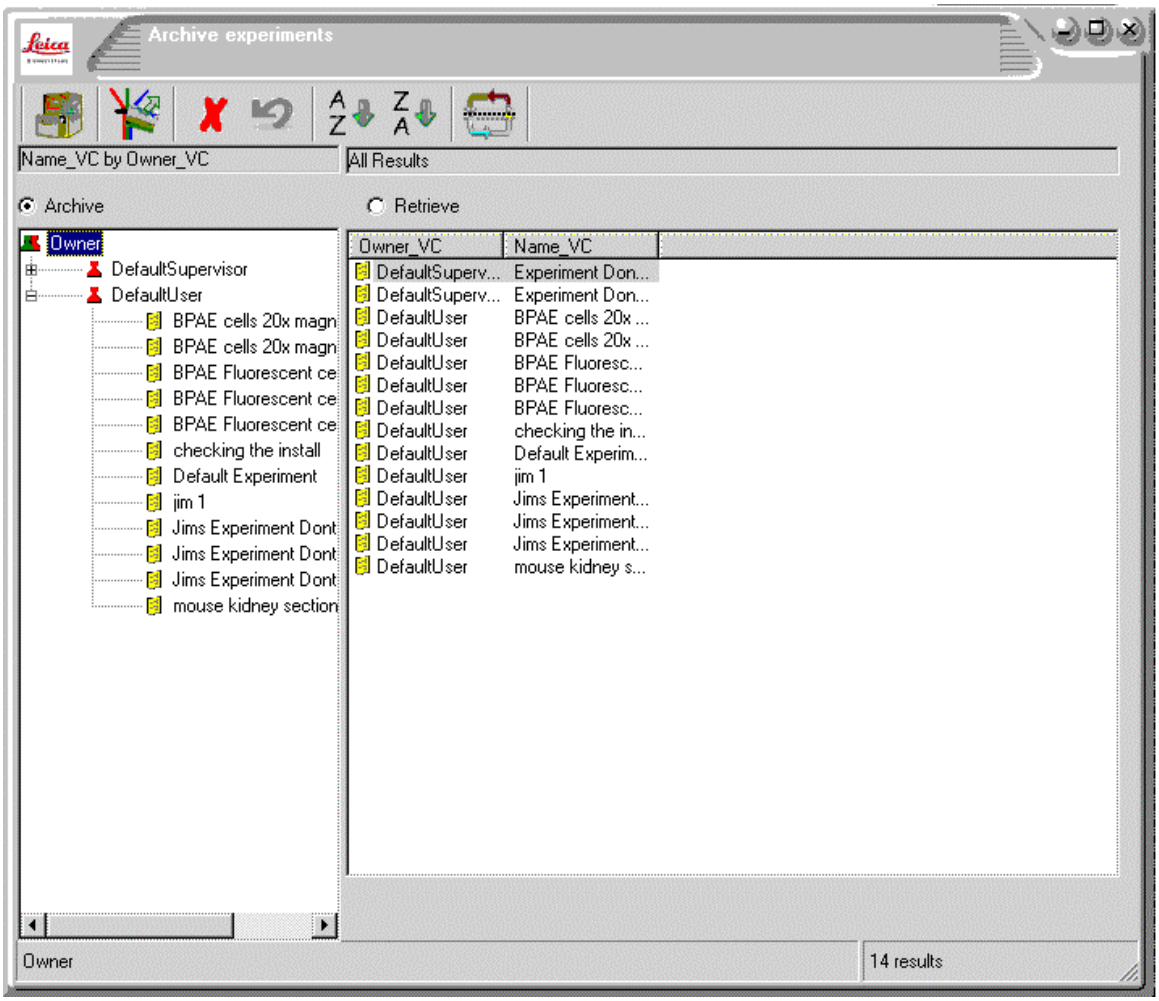


To publish results:

1. Select the publish arrow to allow the current experiment to be processed for publishing as a document.
2. Click **Print Image** or **Print Report** from the **Publish Results** dialog.
3. Select the report format (either 1, 2 or 4 images per page).
4. Drag and drop the images you select into the print image frames.
5. Click **Print**.

Refer to: *Chapter 11: Publishing Experiments*.

Archiving Experiments



The available archiving features include:

- Archiving an experiment.
- Retrieving an experiment.
- Exporting an experiment to a networked PC or removable storage media.
- Importing an experiment.
- Deleting an experiment.

Refer to: *Chapter 12: Archiving Experiments*.

Chapter Four: Setting up an Environment for Experiments

Chapter Overview

This chapter contains the following topics:

- Introduction
- Defining Live Image Acquisition
  - Multi-focus Acquire
  - Adding or Replacing Images
  - Rescale on Images After Acquire
  - Defining the Image Size
  - Setting up the Camera
- Defining the Z-stack
- Selecting Fluorochromes
- Defining Stage Positions
- Defining Time Sequencing Routines
  - Checking the Time Sequence Logic
  - Time Sequence Controls
  - Building a Time Sequence
  - Building a Time Sequence Example Tutorial

Introduction

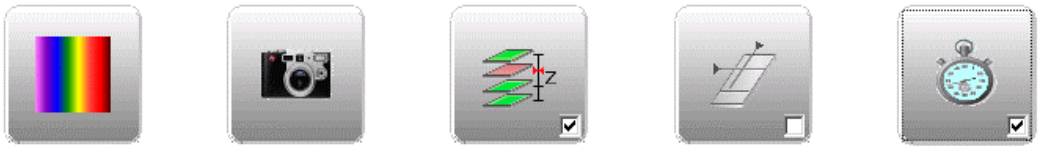
Setting up the environment for your experiment requires you to set up parameters in five areas. These parameters allow you to set up:

- Live images by defining the X/Y image acquisition parameters.
- The Z-stack and choose the spacing between the acquired images.
- Fluorochromes selected from the fluorochrome database, and define the settings to be used for each fluorochrome selected.
- Discrete positions on the sample where you require images to be captured.
- Time sequencing routines used to run the experiment.

This chapter advises you on how to set up each of the parameters and, where applicable, how the parameters interact with each other.

➡ Start the setup process by clicking **Setup** on the Environmental Setup process flow:

The Live Image Setup opens automatically when you click on Setup, and displays a further set of process buttons:



You must check the check box, where applicable, before you can set up that aspect of the environment.

While the Live Image Setup is open you can:

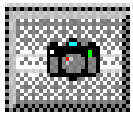
- Open the other dialogs and arrange them on the Desktop.
- Arrange the sequence of the process icons on the screen by right clicking an icon and drag/dropping it to the position required.

Defining Live Image Acquisition

You define the image acquisition parameters by setting the X, Y and Z coordinates, together with setting up the camera and selecting a region for the field of view that will be captured.

➡ To set up the X and Y coordinates click **X/Y**. The **Live Image Setup** dialog is displayed.

➡ Click the **Acquire** button to acquire the image and enter it into the Lab Book. The image is displayed in the **Live Image Setup** dialog.

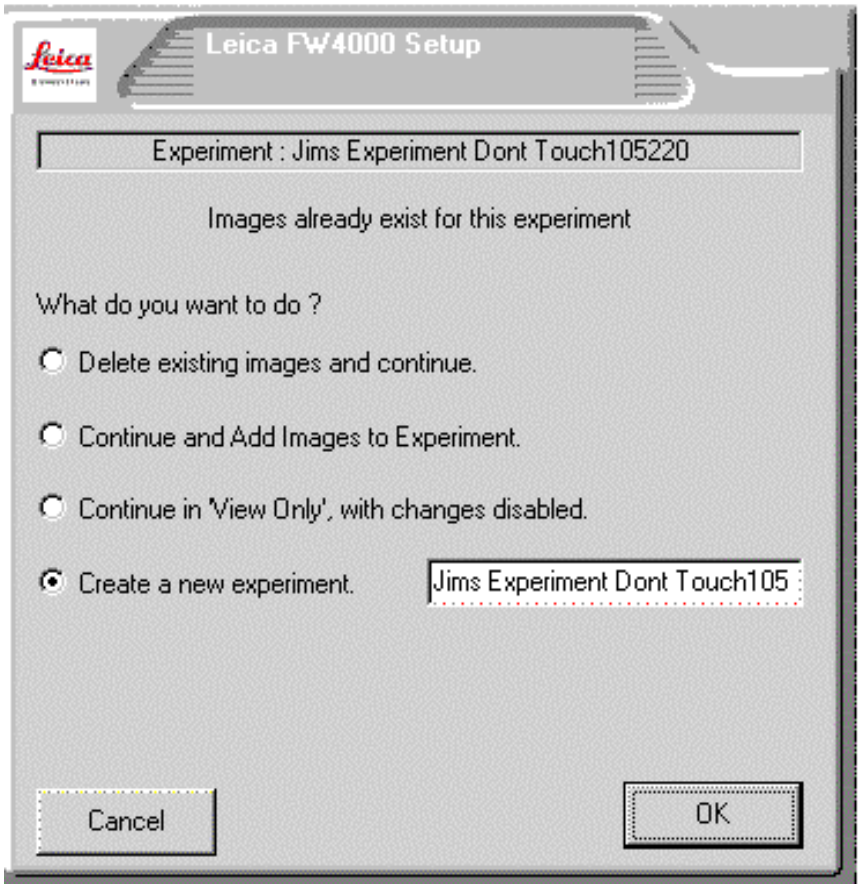


➡ Alternatively, set the image frame and exposure you require, whilst the image is **Live**, and click the **Camera** button.

Refer to: *Chapter 2, Setting General Preferences* for a description on how to cause the Image Viewer to open automatically from the Preferences dialog.

Note: This way of working is aimed at users with basic image capture requirements, perhaps only capturing one image.

When you set up an experiment where images exist already, the Leica Capture dialog appears where you can choose the action to take.


Leica FW4000 Setup
Experiment : Jims Experiment Dont Touch105220
Images already exist for this experiment
What do you want to do ?
Delete existing images and continue.
Continue and Add Images to Experiment.
Continue in 'View Only', with changes disabled.
Create a new experiment.
Jims Experiment Dont Touch105
Cancel
OK

➡ To exit from this stage in the Experimental Setup process, click ⊗ in the top right corner of the **Live Image Setup** dialog.

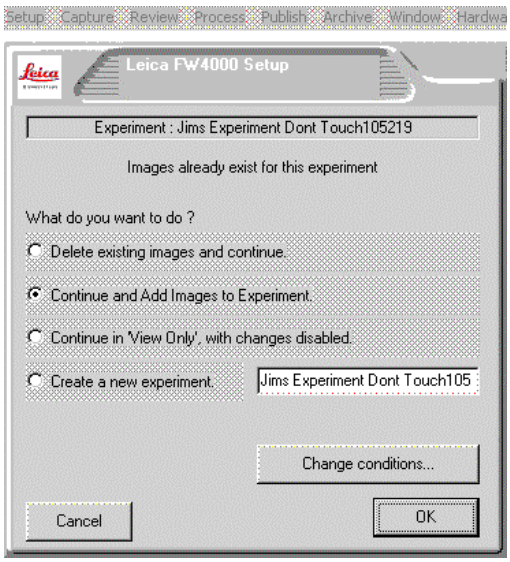
Adding or Replacing Images

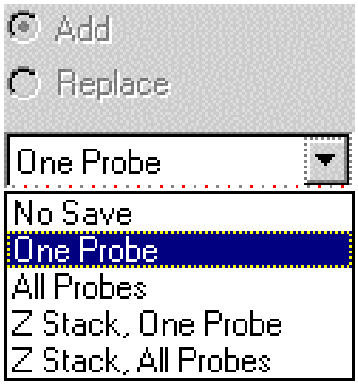
You are able to add or replace images in an experiment at different sessions. The implication is that you can capture images on a tissue sample over a period of time without running that experiment continuously within the **Leica FW4000** application. The time interval between sessions may be defined as weeks, days or hours.

➡ To add or replace a specific image, click either the **Add** or **Replace** radio buttons in the Live Image Setup.

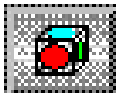
To continue adding images to an experiment:

1. Click **Setup** from the main menu.
2. Select **Continue and Add Images to Experiment** from the **Setup** dialog.
3. Click **OK** to display the **Live Image** dialog. The **Add** radio button is selected automatically, and the **Replace** radio button is 'greyed out'.
4. Select the image capture sequence required from the drop-down in the **Live Image** dialog.





Note: The animated **Cancel Add** status icon appears on the Desktop.

➡ To change the probe in use, click the change probe icon. 

Note: The change probe icon is a short cut to the Setup Fluorochromes dialog.

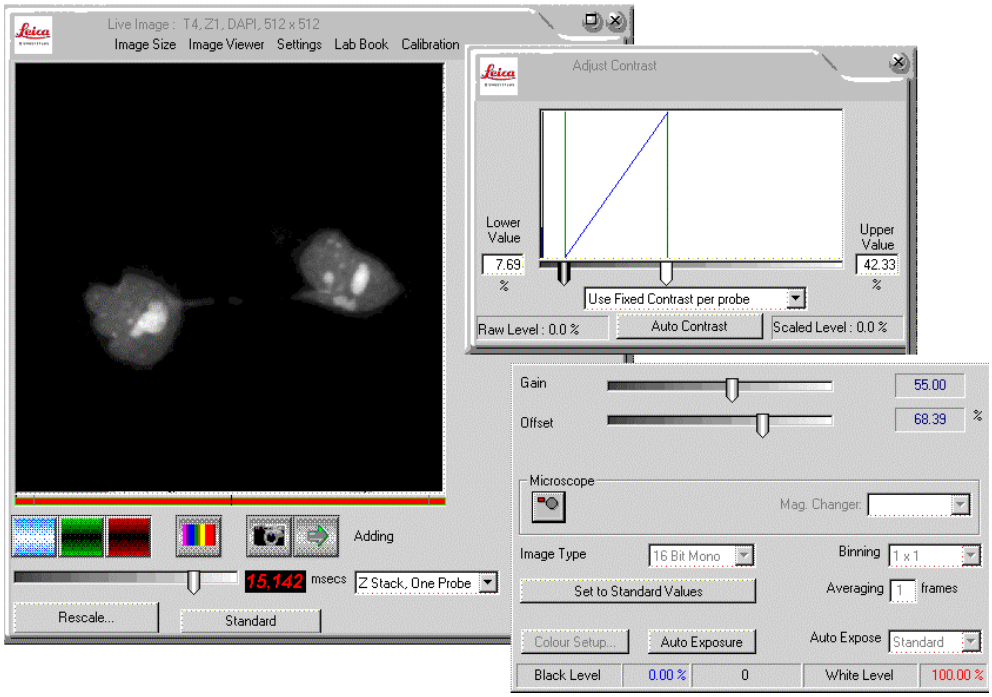
Refer to: *Chapter five, Selecting Fluorochromes* for a description of changing and selecting probes.

Rescale on Images After Acquire

You can set the maximum and minimum grey level values for the scaling of the image, after capture. Subsequent images acquired will be scaled according to your settings. You may set the max and min scaling values by:

- Interaction with the sliding bars.
- Directly typing in the max and min values required.
- Interacting with the grey level histogram.

➡ To rescale an image, click on **Rescale** from the **Live Image Setup** dialog.



Note: The Rescale option will only be available for monochrome images of greater than 8-bit depth.

Defining the Image Size

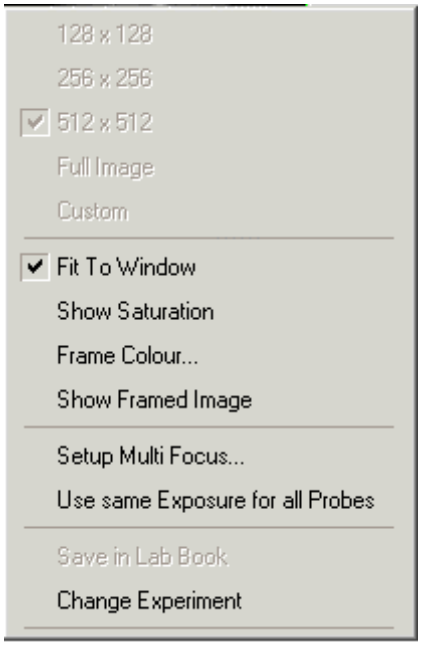
The **main image display** represents the available acquisition area of the CCD camera.

Select a source region as required, for images from the camera. The current values for the image source position and size for the X and Y coordinates are selected when you select the required sub-region.

Note: For cameras with slower image readout rates, using a smaller region for focusing will improve the display update and allow easier focusing of the specimen.

To set a predefined image size:

1. Right-click the mouse in the image area. This displays the **Image Sizing menu.**
2. Click any of the top four menu selections, or click **Custom** and then draw your own image area. When you select **Custom**, the cursor changes.
3. To change the size of a custom-defined image area, click the mouse and redraw the required new image area.

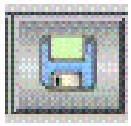


➡ To ensure that you can view the whole image in the image window, right-click the mouse in the image area, and click **Fit to Window**.

➡ To display an indication of whether the displayed image is over-exposed (blue is added) or under-exposed (red is added), right-click the mouse in the image area, and click **Show Saturation**.

- To change the colour of the image frame, right-click the mouse in the image area, and click **Frame Colour**.
- To show only that portion of the image that appears within the frame, right-click the mouse in the image area, and click **Show Framed Image**. See the **Live Image Setup** dialog that follows.

- To set the exposure for one probe and then use this setting for all other probes, right-click the mouse in the image area, and click **Use same Exposure for all Probes**.
- When you select No Save from the capture and save options drop-down menus, any images you capture are not saved. To save the images in these situations, right-click the mouse in the image area, and click **Save in Lab Book,** or click the **Save** icon.



- To start a new experiment, or to delete the images in this experiment and start again, right-click the mouse in the image area, and click **Change Experiment**.

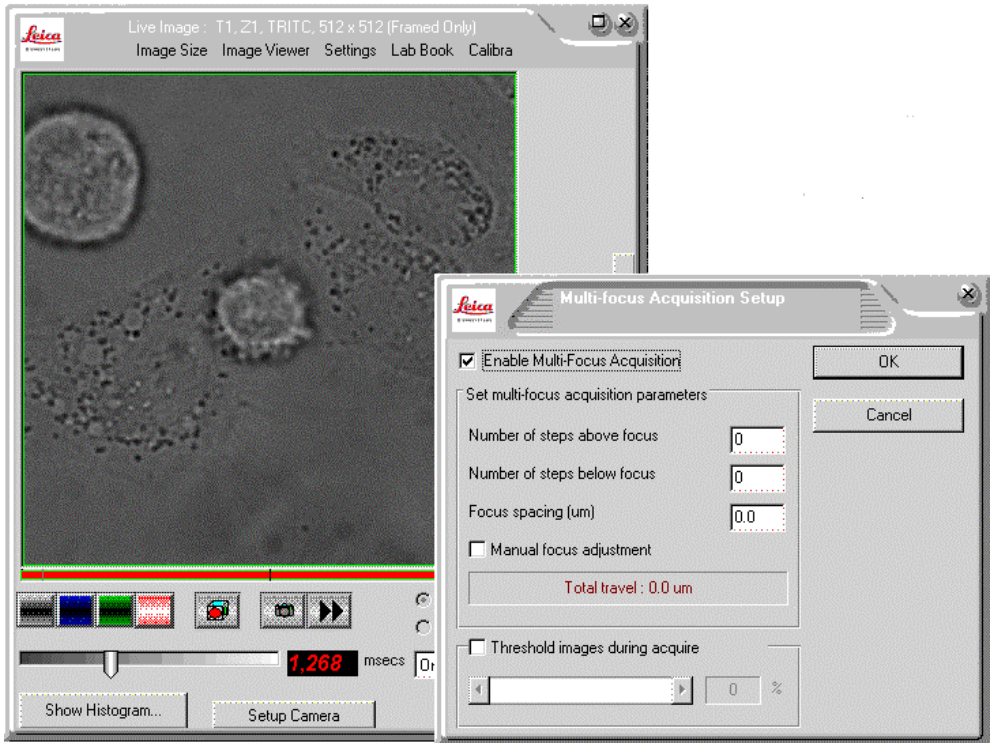
➡ To calibrate an image when you capture it, right-click the mouse in the image area, and click **Calibrate**.

Refer to: *Chapter eight, Calibrating an Image* for further details on calibrating your image.

Multi-focus Acquire

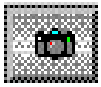
Multi-focus acquire is available in the T, Z and TZ versions of the application but automatic control of the microscope during a multi-focus acquire capture will only be possible with the Z and TZ configurations, which include Z control of an automated microscope.

➡ To use the Multi-focus acquire feature, click on the probe you wish to acquire in multi-focus mode and right mouse click in the image window. Select **Set-up Multi Focus…** from the menu to display the Multi-focus Acquisition Setup dialog.



To acquire the multi-focus image:

1. Check on the Enable Multi-focus Acquisition box.
2. Enter the number of steps below and above your current focal position that you wish to capture images.
3. Enter the focus spacing.
4. If you are working with a manual microscope (without automated Z control), check the **Manual focus adjustment** check box.
5. The system calculates the total travel.
6. Check the **Threshold…** check box if you wish to manually threshold the image during the acquisition.
7. Repeat the above steps for each of the probes you wish to capture in the multi-focus acquisition mode.
8. Click the **Acquire** icon to start capturing for the current probe only (saving in the lab book if required), or the **Capture** icon to start the capture for all probes. The resulting image will be one single image consisting of a maximum projection of all planes acquired.



Note: Use of the Multi-focus Acquire and Z-Stacking features of **Leica FW4000** require that the Z travel limits have been set up in the software. These may be accessed from the **Hardware Setup** menu, 'Initialise focus'.

Setting up the Probes

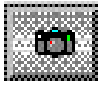
This section describes how to set up the camera for use with each probe.

To set the camera exposure time for each selected probe:

1. Select a probe by clicking the appropriate probe button.
2. Click **Live**. While an image is being captured, the **Live** button is highlighted in red, and the **Shutter** button changes.

3. Move the slide to the required exposure time.

4. Stop the acquiring by clicking **Acquire** again.



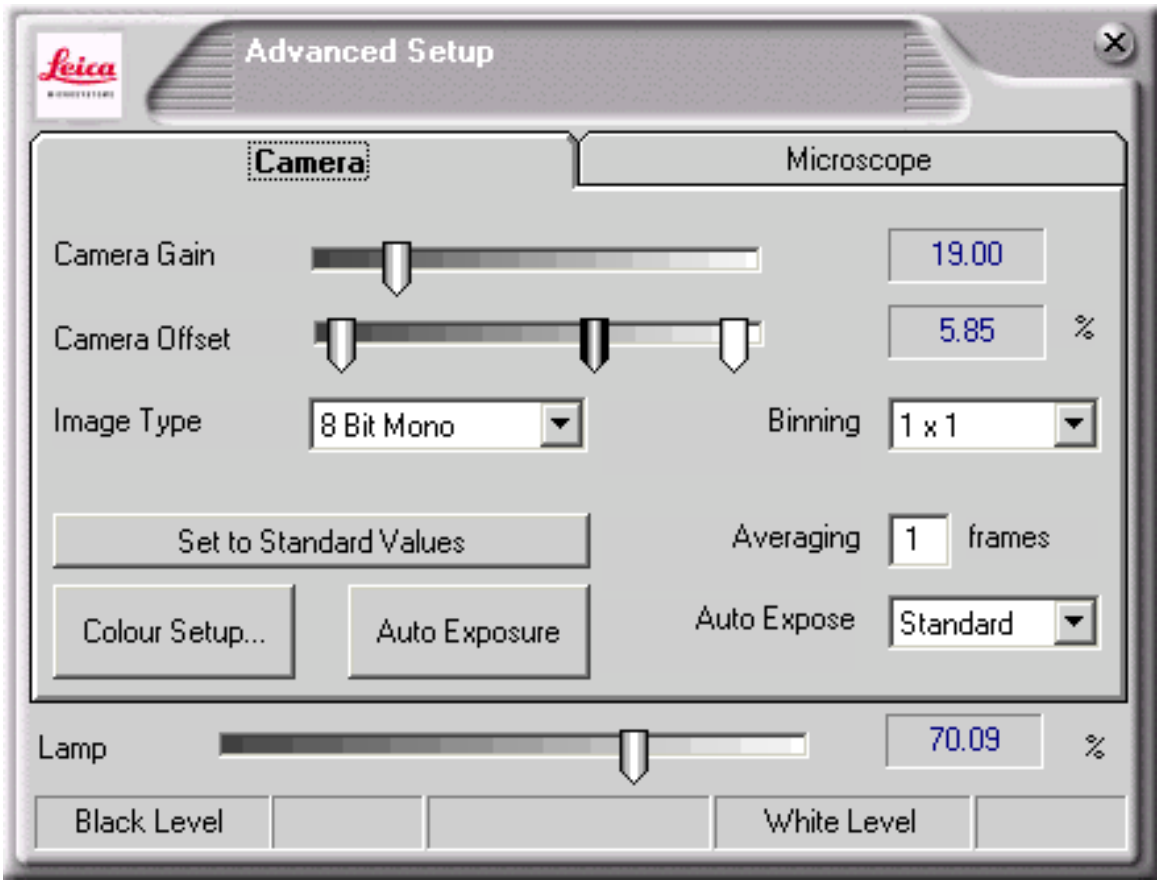
Note: You have a choice here. You can click the **Live** image again to stop being live, because the function changes when you are live (that is, the arrows change to a red square), or you can click on the **Camera** button to acquire the image.

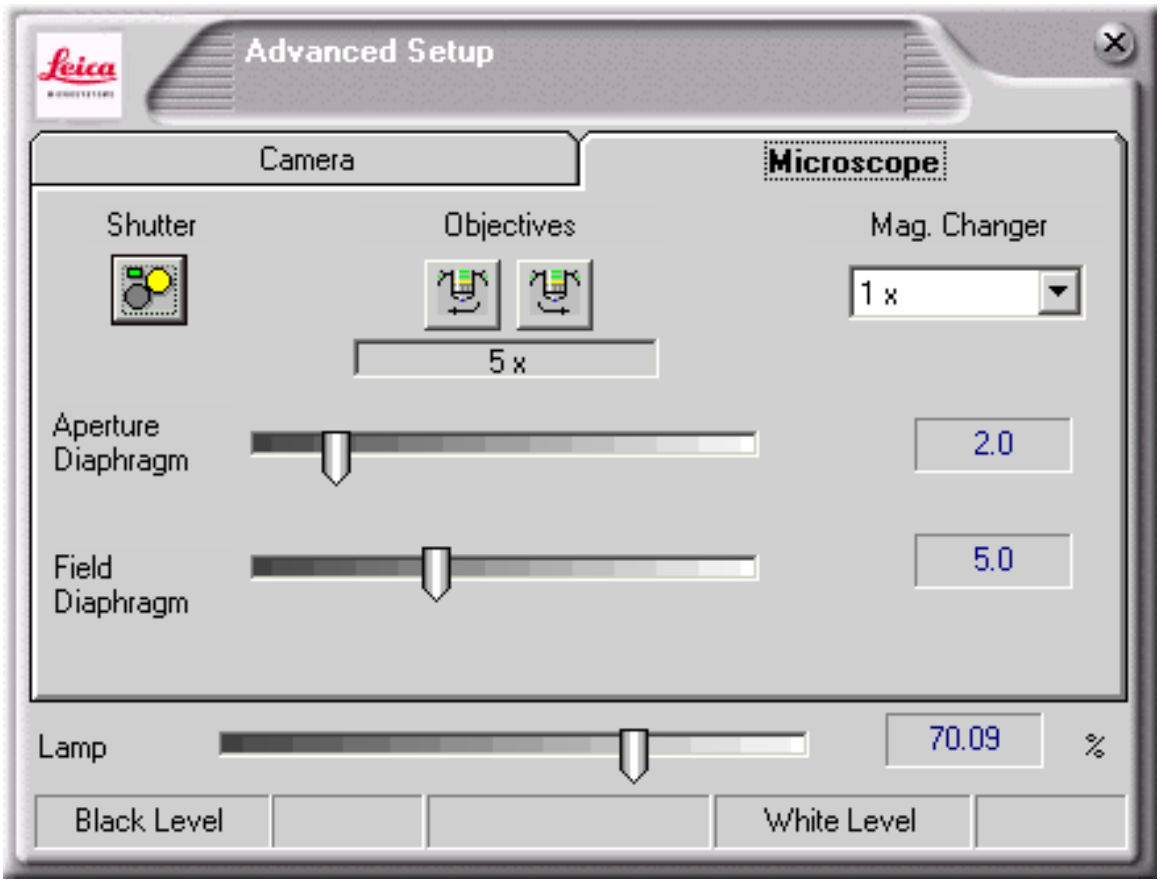
5. Click the next **probe** button and adjust the slider to the required value.
6. You can set up the correct exposure for another probe by repeating steps 1 to 3 but selecting a different probe.
7. Repeat these steps until you have set the exposure time for each probe you wish to use.

Alternatively, you can select a different probe, while a current sequence is running, by clicking another probe button.

Setting up the Camera and Microscope

The Advanced **Setup** dialog image is dependent on the camera in use. To access this dialog, select **Advanced** from the **Setup Live Image** dialog. This dialog has two tabs; one for camera setup and one for microscope setup.





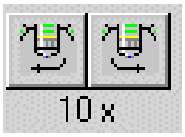
To define the Brightfield lamp brightness:

1. Select the Brightfield probe.
2. Use the **Lamp level** control to specify the brightness of the lamp. Use the slider to change the brightness, or type a value into the text box.

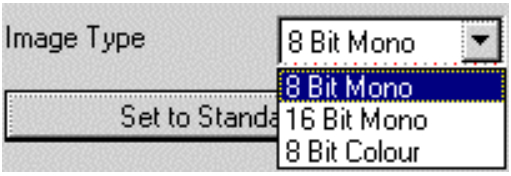
Note: The Lamp level slider is not displayed for other probes.

- Specify a **Gain** value. The current value is displayed in the box to the right of the slider. The increments on the **Gain** control change, depending on which camera is connected.
- Specify an **Offset** value from 0 to 100 percent of the full range available for the current camera. The current value is displayed in the box to the right of the slider.

- Change the **White** signal scaling. The current value is displayed in the box to the right of the slider.
- Change the **Black** signal scaling. The current value is displayed in the box to the right of the slider.
- Click **Set to Standard Values** to reset the camera gain and offset values for the current camera.
- Click **Averaging x frames** to specify the number of images used to create the final image. Multiple frames can be used to prevent noise.
- To set objective magnification level, use the next and previous **Objective** buttons.



- **Binning** represents the binning mode of the image.
- The **Image Type** represents the bit depth of the image. You select the appropriate value from the drop-down menu.

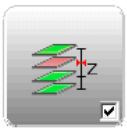


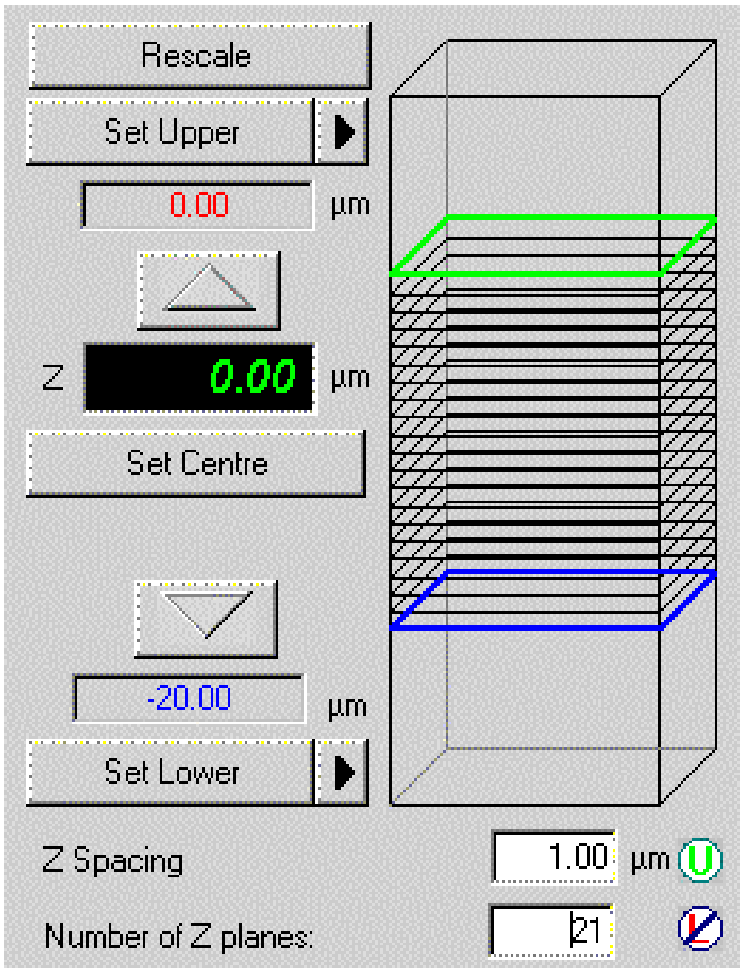
Defining the Z-stack

The Z-stack allows you to acquire images at different focus positions automatically by setting the upper and lower limits for the Z-position.

Note: The Z-stack is a **Leica FW4000** product option that is only available if you have purchased the **FW4000Z** or **FW4000TZ** modules.

To define the Z-stack, click **Z** in the **Process** flow or the **Live Image Setup** dialog. The **Z Stack** dialog is displayed.





To set the upper limits for the Z position:

1. With the mouse, drag the green plane to the desired upper limit value.
2. Set the upper limit value by clicking **Set Upper.**
3. Drag the green plane to the desired lower limit value.
4. Set the lower limit value by clicking **Set Lower**.

Tip: The recommended way of working is to have a live image visible in the XY dialog. Use the focus control of the microscope, to see when you have found the upper limit of travel you wish to define, and then click the **Set Upper** button. Repeat this process for the lower limit.

Type the **Number of Z Planes** required in the box provided. The **Z Spacing** is calculated and displayed automatically.

5. Click the **Z Spacing lock** or **the Number of Z planes lock** button.

Note: The purpose of the locks is to fix the number of planes and calculate the Z planes or vice versa. Both the **Z Spacing lock** and the **Number of Z planes lock** may both be off but only one may be on at any time.

6. Click the up and down **arrow** buttons, to move the focal plane one plane per click, until the value you require is displayed.
7. Click **Set Upper** or **Set Lower** to confirm the focal plane value.

To display the image at either end of the Z Plane range, click the relevant **Set Upper** or **Set Lower** arrow.

The focal plane colour changes as you click either of the two arrows:

Focal plane	Colour
Upper	Red
Lower	Blue
Current	Green

➡ When you set the upper or lower values such that they are not visible on the scale within the cube, click **Rescale**. This action repositions the family of Z planes back in view and scales them to give a good graphical representation within the cube.

➡ To define the current position of the Z-stack as the centre of the stack, click **Set Centre**.

Note: Once you have decided on the mid position (typically the most in focus image or the zero position set on a DM microscope) and clicked **Set Centre**, the number of planes required will be divided above and below this centre point.

Note: To use any feature in **Leica FW4000** requiring automated movement of the microscope, you must ensure that the upper and lower focus limits are set. This can be done by selecting **Initialise Focus** from the **Hardware Setup** menu.

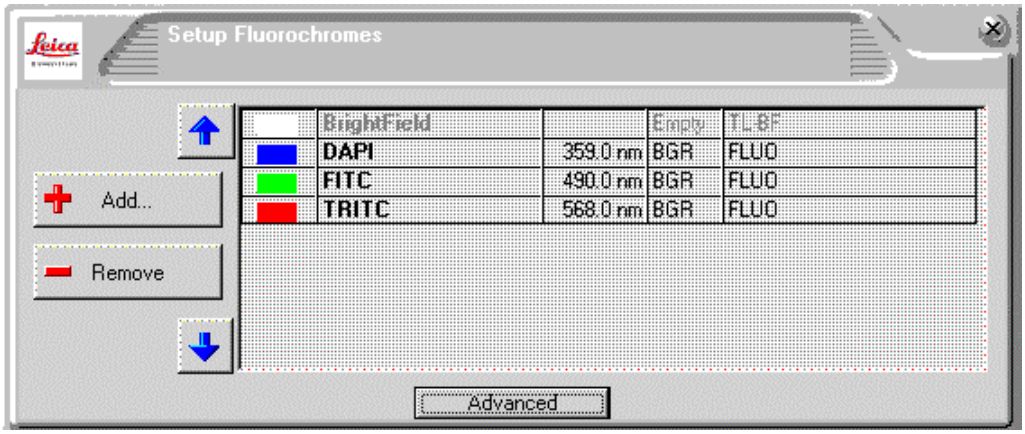
Selecting Fluorochromes

This stage in setting up the parameters for your experiment allows you to set up the fluorochromes that are relevant to your experiment.

The **Setup Fluorochromes** dialog allows you to:

- Select the fluorochromes used by each image.
- Add fluorochromes.
- Create fluorochromes.
- Modify fluorochromes.
- Specify the colour used to represent each fluorochrome.
- Select the sequence in which the fluorochromes are displayed on dialogs and captured during acquisition sequences.
- Define the wavelength of light used to capture an image of a particular fluorochrome.

➡ To select Fluorochromes click λ. The **Setup Fluorochromes** dialog is displayed.



The **Select Fluorochromes** dialog (see the screen below) allows you to organise the fluorochrome database by adding and deleting fluorochromes and entering detailed information.

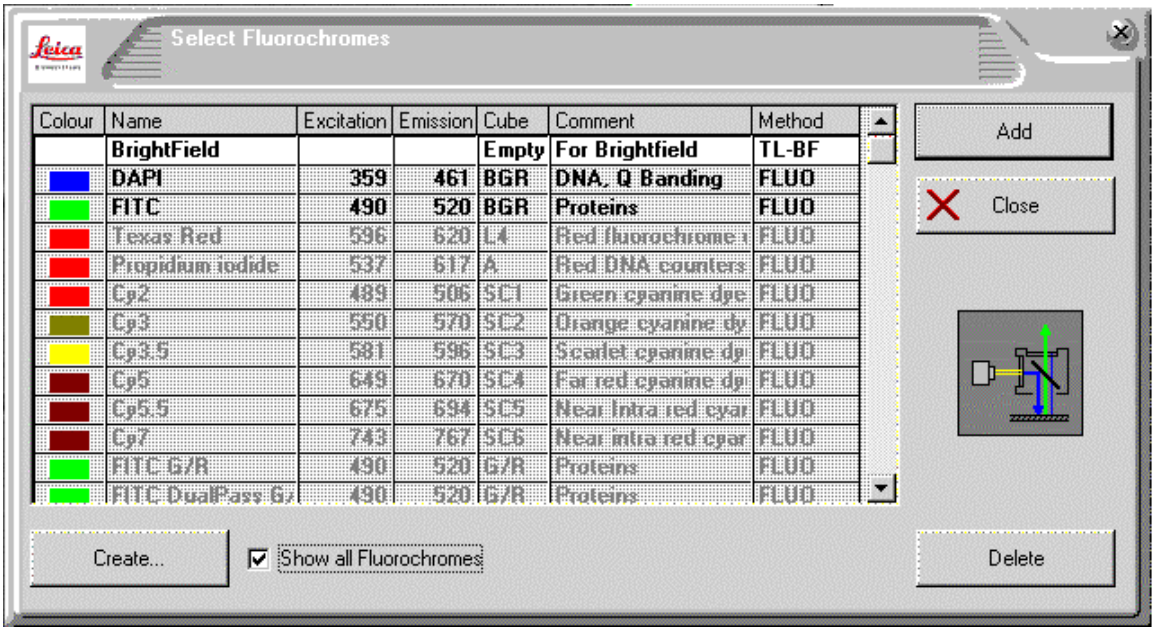
Available fluorochromes are listed in the centre area of the dialog where detailed information for the selected fluorochrome is also displayed.

➡ To change the sequence of image display in the Image Setup, use the **Move Up** and **Move Down** blue arrows to physically move a fluorochrome in the list.

➡ To exit from this stage in the Environmental Setup process, click the ⊗ button in the top right corner of the **Setup Fluorochromes** dialog.

To add a fluorochrome to the active list:

1. Click **Add**.



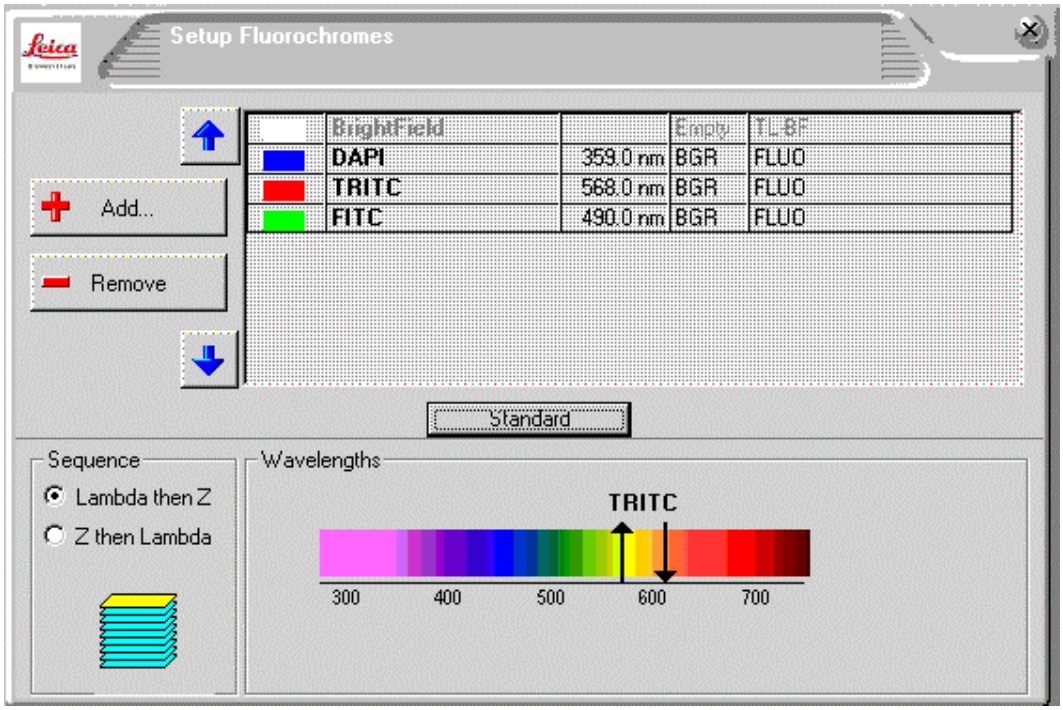
2. Double-click **Name** for the fluorochrome you wish to add to the **Setup Fluorochromes** dialog, or highlight the fluorochrome required and click **Add**.
3. Click **OK**, or **Cancel** to cancel the operation.

➡ To display a predefined list of default fluorochromes from which you can select, check the **Show all Fluorochromes** box.

➡ To delete selected fluorochromes, click **Remove**.

To change the sequence of image capture:

1. Click the **Advanced** button in the **Setup Fluorochromes** dialog, to display the **Setup Fluorochromes Advanced** dialog.

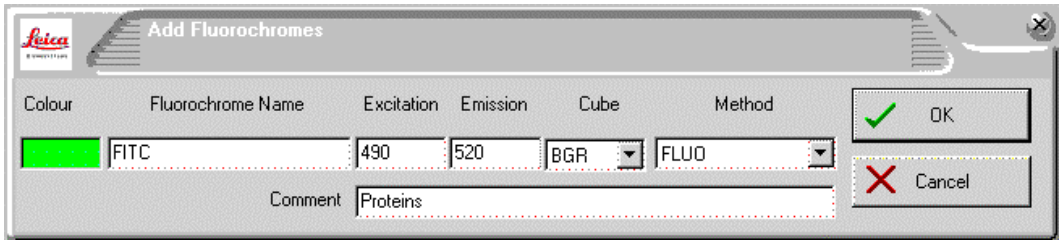


2. Toggle between the **Lambda then Z** and **Z then Lambda** radio buttons.
   This radio button changes the order in which the images are captured. For example, **Lambda then Z** will go through the stack capturing all the DAPI images followed by all the FITC images, **Z then Lambda** captures DAPI, FITC, DAPI and FITC.
3. Click the Standard button to return to the Set Fluorochromes dialog

Note: The wavelength boundary for the fluorochrome selected in the list of fluorochromes is highlighted in the Advanced section of the dialog.

To create a fluorochrome:

1. Click **Create** in the **Select Fluorochromes** dialog. This displays the **Add Fluorochromes** dialog.

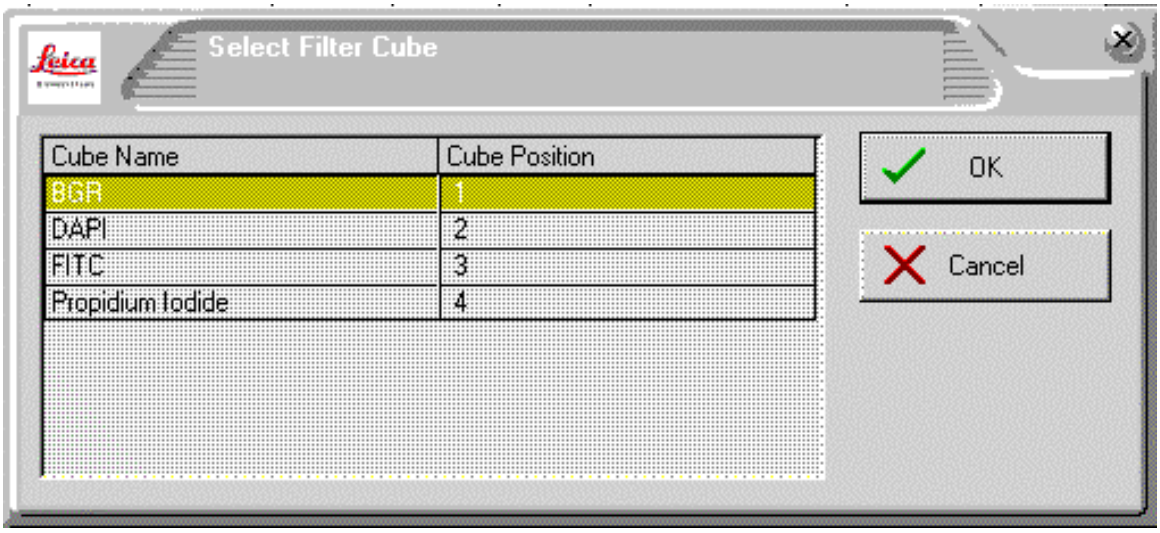


2. Click in the **Colour** field. Choose a colour from the **Colour Palette**, and click OK to return to the **Add Fluorochromes** dialog.
3. Type a **Name** for your fluorochrome.
4. Edit the values contained in the **Excitation** and **Emission** boxes.
5. Choose a **Cube** from the drop-down menu available in the Cube field.
6. Type a **Comment**, if required.
7. Select the **Method** required for this fluorochrome (for example, Brightfield or Fluorescence).
8. Click **OK** to add the new fluorochrome to the list of fluorochromes, or click **Cancel** to cancel the operation.

The **Select Filter Cube** dialog allows you to specify or modify the fluorochrome filter used in each filter position. The filter associated with each filter rotator position is established by automatic examination of the microscope.

To set up a filter cube:

1. For each filter cube, double-click the required fluorochrome from the list of appropriate fluorochromes in the **Select Fluorochromes** dialog. This displays the **Select Filter Cube** dialog.



2. Define all the filter cubes as appropriate.
3. Select the filter cube you would like to use by clicking on the **Cube Name or Cube Position** field.
4. Click **OK**, or **Cancel** to cancel the operation. The selected filter cube is added to the fluorochrome list.

➡ To change the colour representation of a fluorochrome filter, double-click the **colour** for the fluorochrome filter you wish to change. Select the appropriate colours from the extensive colour database displayed, or design your own colour. Click **OK** to apply the new colour.

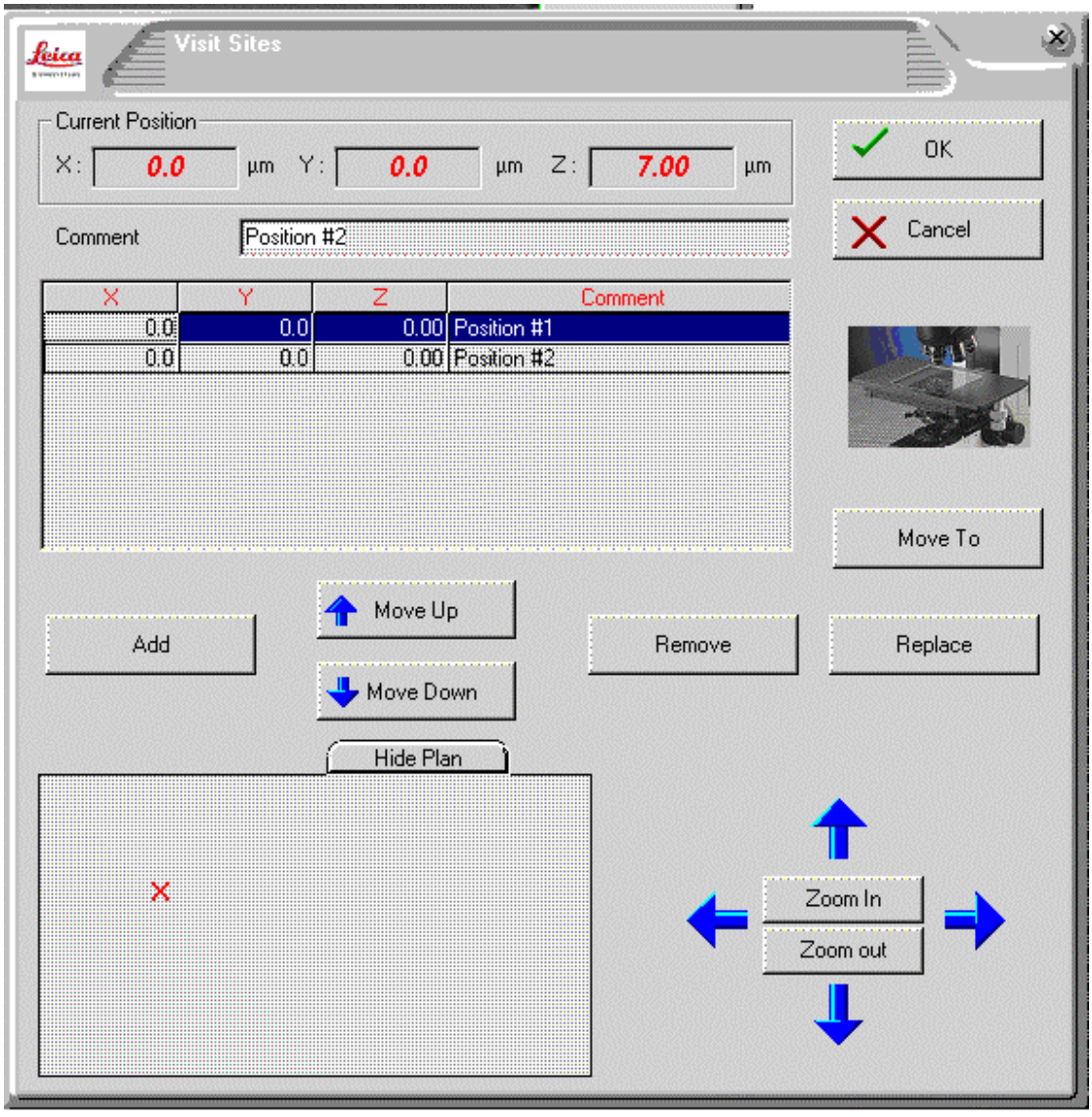
➡ To change the defined filter for a fluorochrome in the default list, double click on the filter name (for example, RGB in the **Select fluorochromes** list). From then on, any new experiment will automatically be set with the correct filter position for the defined microscope, and filter positions will only require changing if filters are added or removed from the microscope.

Defining Stage Positions

The **Visit Sites** dialog allows you to pre-set the position of the stage.

Once you have set up the stage positions, you can use this dialog to move between these pre-set positions. You can also save a list of positions for subsequent re-use.

➡ To define stage positions, click **N**. The **Visit Sites** dialog is displayed.



The **Current Position** area at the top of the dialog displays the current position of the stage.

Tip: You are recommended to display the **Live Image Setup** and **Visit Sites** dialogs together. Then you can use the live image as a view, the co-ordinates of which are recorded in the **Visit Sites** dialog. Taking this approach will help you to design an effective stage plan.

To add a position to the stage position list:

1. Locate the area of interest.
2. When you have located the area of interest and adjusted the focus, return to the **Visit Sites** dialog.
3. Select the **Comment box** and type the name for the new stage position to identify it at a later time.
4. Click **Add**.
5. Click **OK**, or **Cancel** to cancel the operation.

To replace a position in a stage position list:

1. Locate the area of interest.
2. When you have located the area of interest and adjusted the focus, return to the **Visit Sites** dialog.
3. Select the position you want to modify by clicking anywhere in this row.
4. Click **Replace**.
5. If you want to add a comment to help you identify this position later on, type a comment into the **Comment** box of the dialog before clicking **Replace**.

➡ To remove a position in a stage position list, select the position you want to remove by clicking anywhere in this row. Click **Remove**.
To remove all positions from the stage positions list, you will need to select each one in turn and click **Remove**.

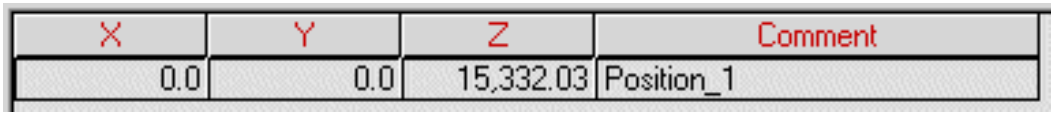
➡ To replace a position in a stage position list select the position you want to replace by clicking anywhere in this row. Click **Replace**.
To replace all positions from the stage positions list, you will need to select each one in turn and click **Replace**.

➡ To move the stage from one position to the currently selected position, click **Move To**.

➡ When you have completed all the required activities, click **OK**, or **Cancel** to cancel the operations.

As you define new positions, that position is shown as a small red cross on the stage plan. You can move round the stage plan by using the **Zoom In** and **Zoom out** buttons. When the cursor is moved over a red cross, a comment on that stage is displayed as a pop-up message.

➡ To add or change a stage comment, highlight the **Comment** field and type the text you require.



X	Y	Z	Comment
0.0	0.0	15,332.03	Position_1

➡ To exit from this stage in the Environmental Setup process, click the **OK** button in the top right corner of the **Visits Sites** dialog.

Defining Time Sequencing Routines

Note: The **Time Sequencer** is a **Leica FW4000** product option that is only available if you have purchased the **FW4000T** or **FW4000TZ** modules.

A time sequence is a list of instructions that controls the acquisition of images during an experiment. The time sequence is defined by using the Time Sequence Builder. The sequence is built from a list of elements that are accessible from control buttons available via the **Time Sequence Builder** dialog.

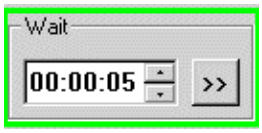
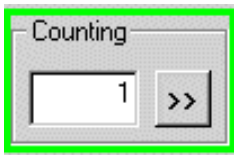
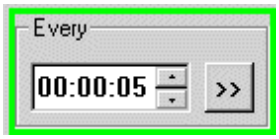
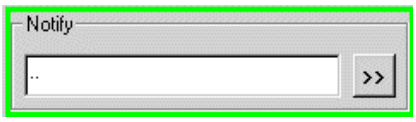
An automatic image capture tool allows you to set up and store all the parameters required to acquire a number of images automatically. Once the sequencing routine has been set up, the capture and processing of images is a fully automatic process.

Each time a routine is run, any or all of the steps can be included. For example, skipping one time interval when images are defined for a given frequency of capture, to another frequency of capture. You amend a sequence by inserting or deleting steps, or amending step parameters.

Each element in the list may be edited by the controls that will appear on the left side of the form when the element is selected by cursor keys or by being touched by the mouse pointer.

The controls you can use to build your time sequence include:

Time Sequence Builder components	**Action**
IMAGE command	Instructs the software to perform the defined experiment
	Runs for a defined period of time: ♦ Until stop ♦ Until a specified time ♦ For a period of time

	Waits for a period of time before executing the next.
	Sets the number of times the last action or nested sequence is repeated.
	The time frequency executing the last action or nested sequence.
	Free-format message to be displayed at this point in the overall sequence.
Move Up Move Down	These change the position of a highlighted element in the time sequence.
Pause command	The sequence pauses until it is restarted.
Move right command	Moves a selected number of instructions to the right. This action will change the overall sequence, as you will create a nested sequence.
Move left command	Moves a selected number of instructions to the left. This action will change the overall sequence, as you will remove a nested sequence.
Delete command	Deletes a selected sequence component.

Note: You should refer to the list of Time Sequence builder actions available when you build the time sequences later in this chapter.

You can set up a time sequence that has fixed times of day in some of its steps. A sequence that says Run Until 14:00:00 is reasonable if the

experiment actually started at 13:50, but becomes much longer (and unreasonable) if the actual experiment start time is later than 14:00.

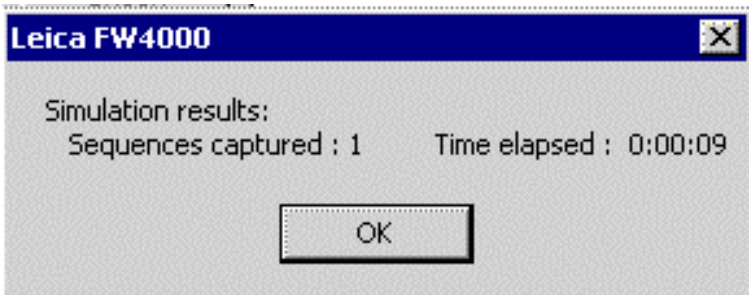
Tip: Use fixed times of a day when building a time sequence with circumspection.

A warning is given at run time when the experiment encounters a fixed time of day that has already passed (the system can only assume that the experiment is intended to use the next occasion that this time occurs, which could be the following day). Special circumstances may require fixed times for experiments, but generally times relative to the start of the experiment are preferable wherever possible.

When times are entered in the various editing boxes the system will report these in a friendly phrasing ("5 minutes" instead of 00:05:00) but on the experiment log the times will be in the 24 hour clock representation, which is how they are held on the database.

Checking the Time Sequence Logic

Clicking the **Check** button allows you to see what will happen with a particular Time Sequence by simulating the capture process you have defined. The results are displayed with the anticipated outcome:



Note: This successful result could be invalidated if you change the experiment setup later to have a very long exposure time, with the 5 second cycle too short to allow each IMAGE operation to complete.

The metronome will, in this case, miss a beat. Therefore, you are advised to check the time sequence after you have set up the imaging characteristics.

It is possible for you to build sequences that do not have sensible meanings. The **Check** button:

- Scans a time sequence to identify any blocks that do not actually capture any images. (This situation usually results when steps are deleted in a sequence without taking care of the left over indentation.)
- Corrects (some of the easier) indentations to make meaningful sequences.
- Identifies expressions that are incomplete (for example, **Run..**) where no time limit or stopping time has been set.

Time Sequence Controls

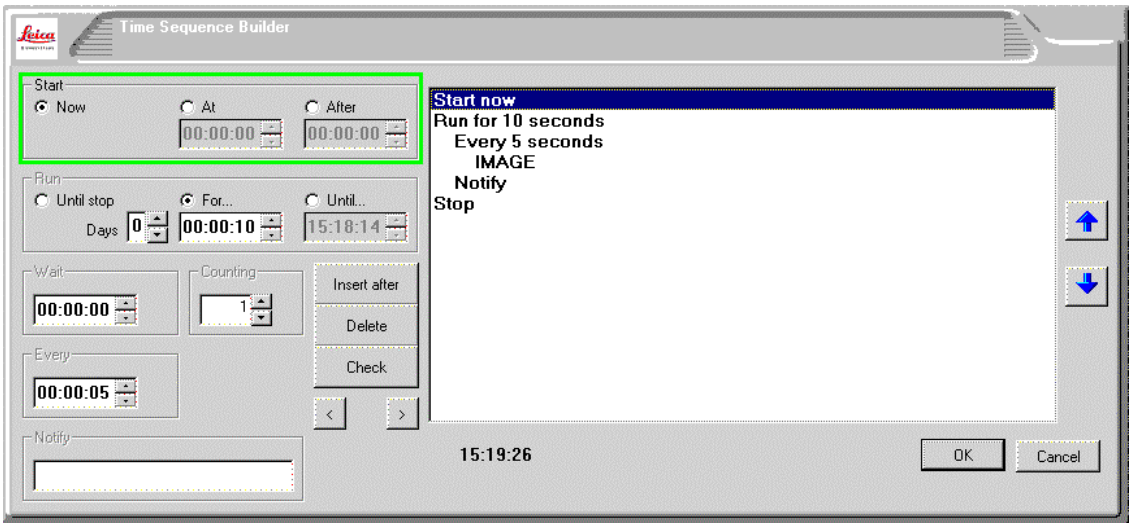
Consider that the system has one referee (a *Run for 10 minutes* element) and one metronome (an *Every 5 Seconds* element). The metronome is responsible for causing things to happen, the referee blows the whistle to signal at the time to Stop.

There is only one referee and only one metronome in the system, so a later **Run** or a later **Every** element will override the effects of an earlier one.

Two controls are required as the metronome has to be set to beat at a regular interval, while the **Wait** timers can be set individually to any time period desired. This gives more control to the experimenter but requires a little more thought in setting up the sequence.

Building a Time Sequence

To set up the time sequencing, click **T**. The **Time Sequence Builder** dialog is displayed.



To build a time sequence:

1. Design and write down the proposed script for your experiment, taking into account all the components listed in the table above.
2. Click **Insert After**. Select the appropriate action and set the time period, duration or count.

3. Repeat step 2 until you have compiled the complete sequence that represents your experiment.

Note: As you highlight the time sequence elements, the appropriate element controls (displayed on the left of the Time Sequence Builder window) are activated.

Therefore, when you wish to edit an element in the time sequence, highlight the element and make the changes by using those controls, or by typing into the text box.

Note: You must change non-variable time sequence elements by insertion or deletion.

4. Click **OK,** or **Cancel** to cancel the operation.
5. If the logic of the time sequencer is flawed, a message is displayed; before you can move to the next stage of your experiment you must correct any errors.

➡ Alternatively, you can check the logic of your time sequence by clicking **Check**. Again, if the logic of the time sequencer is flawed a message is displayed, however, before you can move to the next stage of your experiment you must correct any errors.

➡ Click **Delete** to delete the highlighted time sequence commands.

➡ Click the left pointing arrow to move a command to the left.

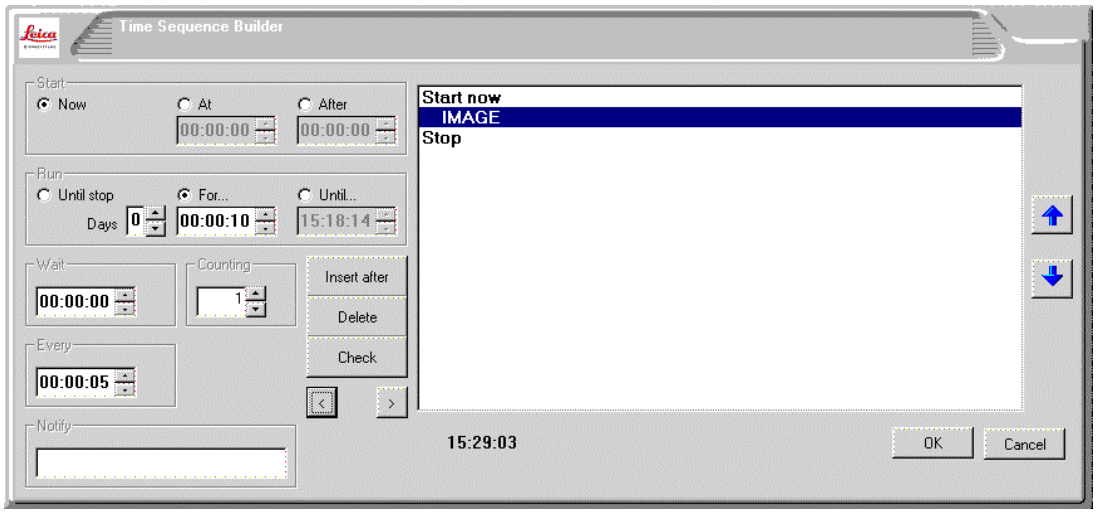
➡ Click the right pointing arrow to move a command to the right.

Note: Notice that toggling between the **L** and **R** buttons allows you to adjust the indenting (and nesting) of sub sequences in your time builder.

Building a Time Sequence Example Tutorial

This tutorial will guide you through building a complex time sequence. We will start with a simple example and add time sequence elements and build a complex sequence.

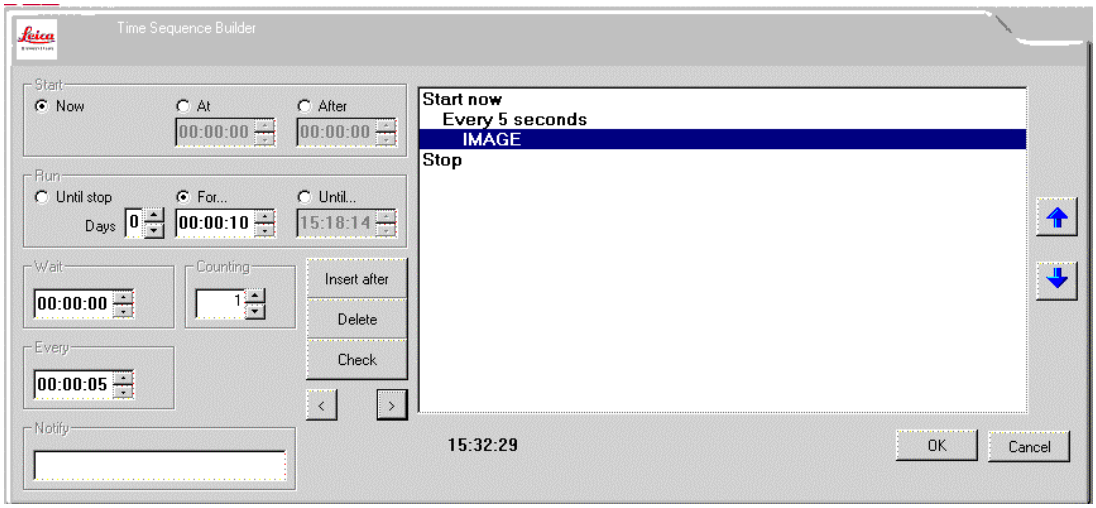
➡ Build the simplest possible time sequence that contains the elements **Start now**, **IMAGE** and **Stop**.



This sequence starts as soon as the Capture button is pressed, runs for 10 seconds and takes images at five-second intervals. What IMAGE does will depend on other settings for the experiment. It may be a single frame from the camera, or a complete Z-stack of many planes in several fluorochrome probes.

In this example the **Start** element is selected, so you can set a Start Time of day by selecting the **At** button, or you can set an initial delay by using the **After** button.

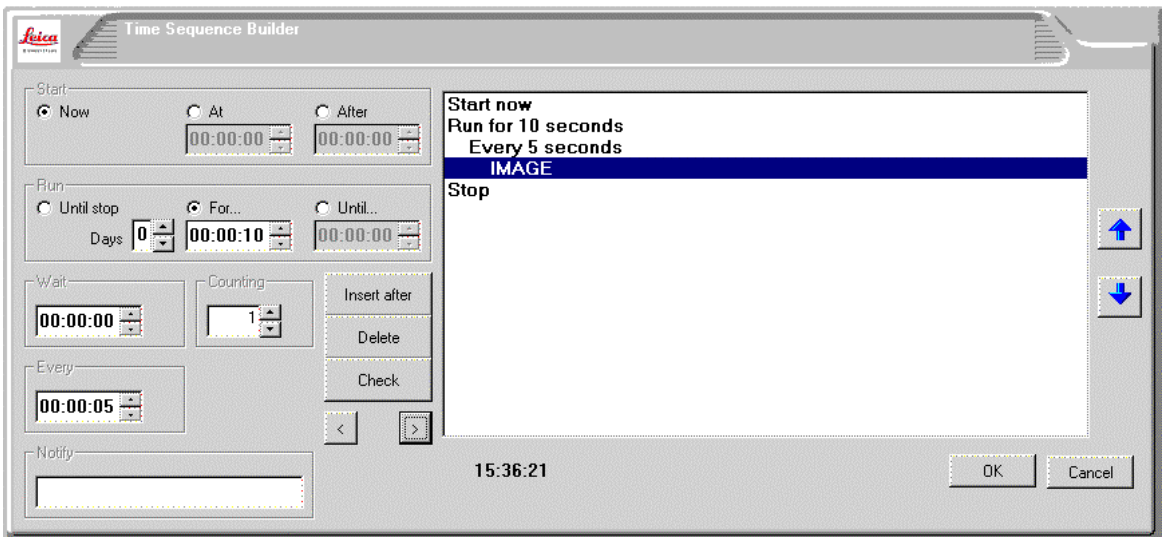
➡ Add the **Every** element into the previous example.



The **Every** element causes an IMAGE operation (which could be a single frame or a complete multiprobe stack) to take place every five seconds.

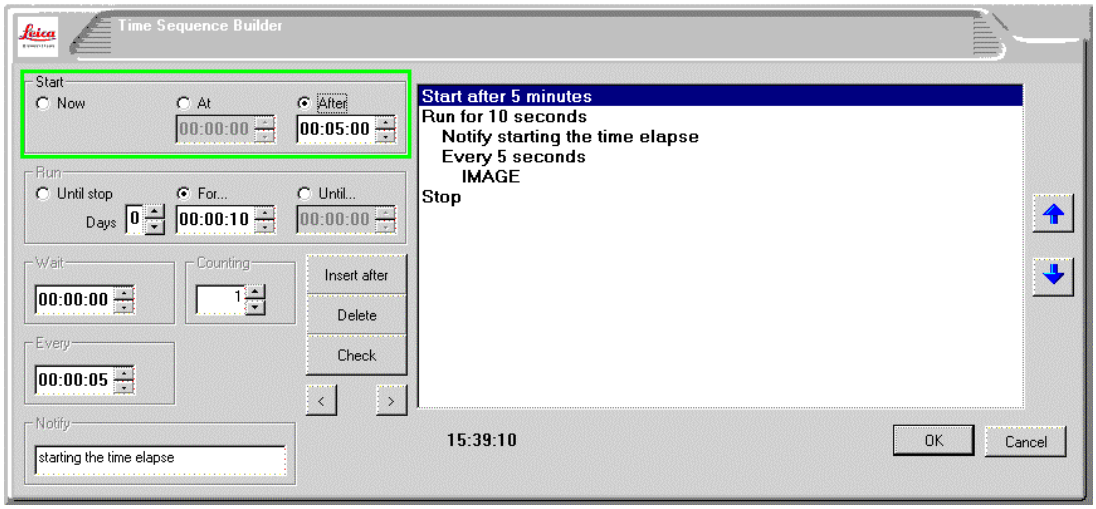
Note: This is a large experiment! There is no restriction on how long the experiment should run, so a maximum elapsed time of 24 hours is assumed.

➡ Add a **Run** element. The example restricts the run to 10 minutes.



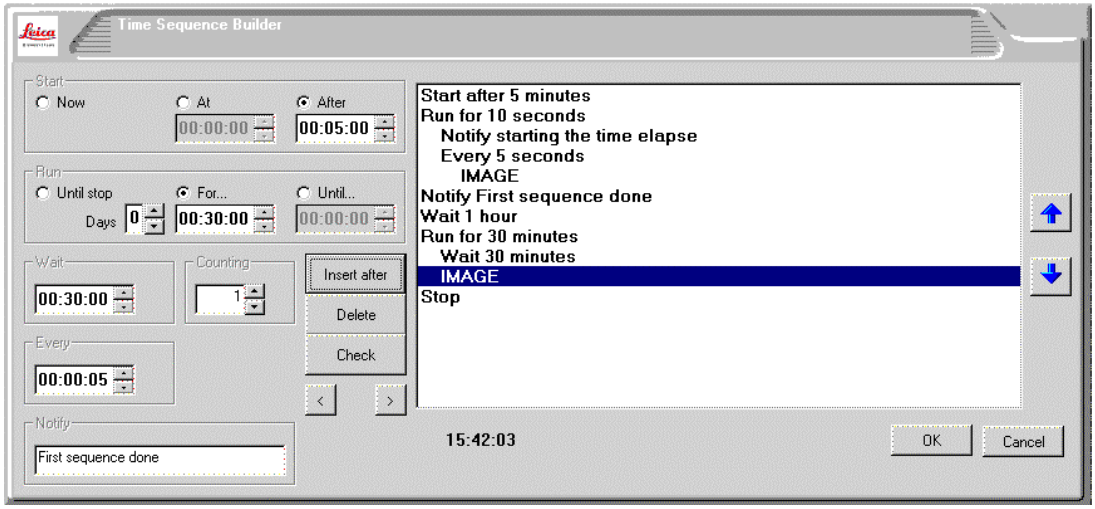
Note: How the elements are indented. This means that the **Every five seconds** instruction keeps control, doing an IMAGE every five seconds, and will not release control until the 10-minute time period is achieved.

➡ Add a **Notify** element to produce a message on the log and on screen during the image acquisition process.



Note: There will be only one occurrence of the Notify message because of the indenting protocols. See the previous Note.

➡ Add two **Run** blocks, one after the other, with an hour's wait in between.

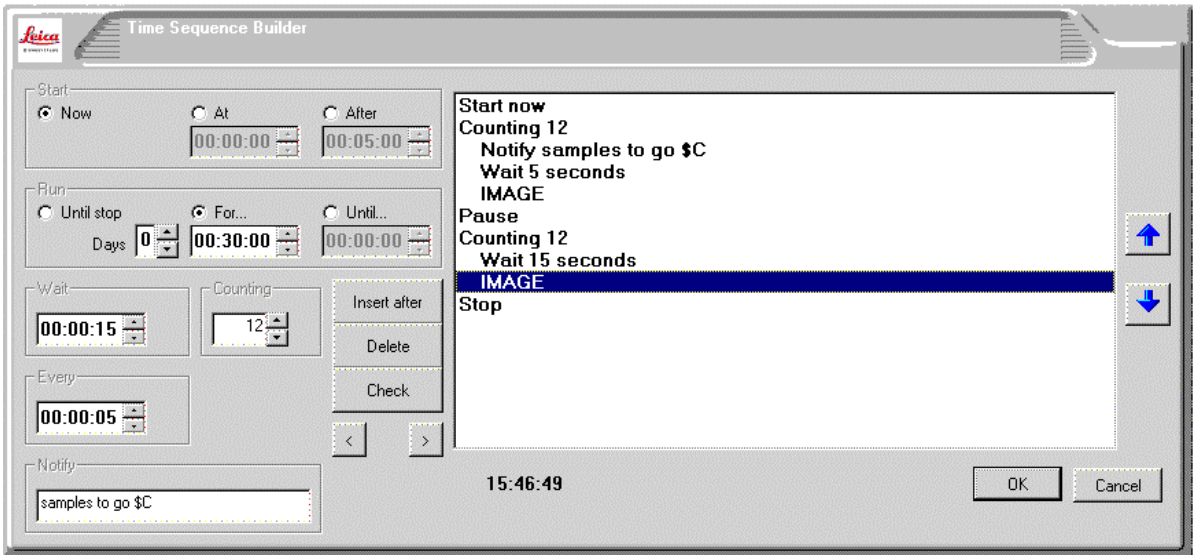


The first Run block takes images at a higher rate than the second. The **Notify** element displays a message “First sequence done.” after the first Run block has finished.

Note: Notice that the **Notify** element is not indented. If it were aligned with the IMAGE element above (by pressing the **R** button twice) then the **Notify** would be part of the **Every 5 seconds** step, and there would be many occurrences of the message on the log. Sometimes this is desirable (see below), but not in this case.

The Notify element displays the given message on the log, but will replace an occurrence of the character sequence **$T** with the current time, so you can produce additional time stamps during an acquisition.

➡ Build an example that takes twelve samples with five second waits in between them, then pauses until you tell the sequence to continue. Follow with the acquisition of twelve further IMAGE samples with fifteen second waits in between each image capture.



Note: The first **Counting 12** element is subtly different from taking twelve samples every fifteen seconds, because the time to take each sample will extend the duration of the experiment.

Note: The character sequence $C in a Notify; during the acquisition this will be replaced by the current unexpired Count in any Counting block.

Note: In this example there is no metronome. Consider that you are using an egg timer that is started after each IMAGE operation and which holds up the experiment for the specified time.

Chapter Five: Capturing Images

Chapter Overview

This chapter contains the following topics:

- Capturing Images Overview
- Using the Image Capturing Tools

Capturing Images Overview

The image capturing sequence performs the set of programmed time sequences you designed when setting up the time sequencing program.

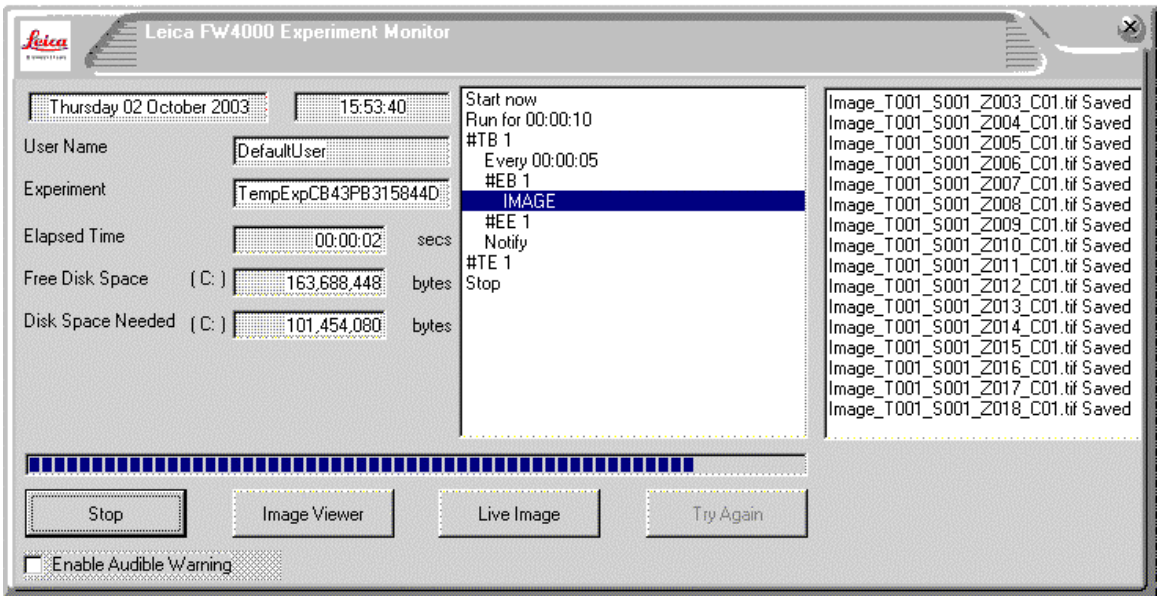
Refer to: *Chapter 4, Defining Time Sequencing Routines*.

The key features of the image capture process are:

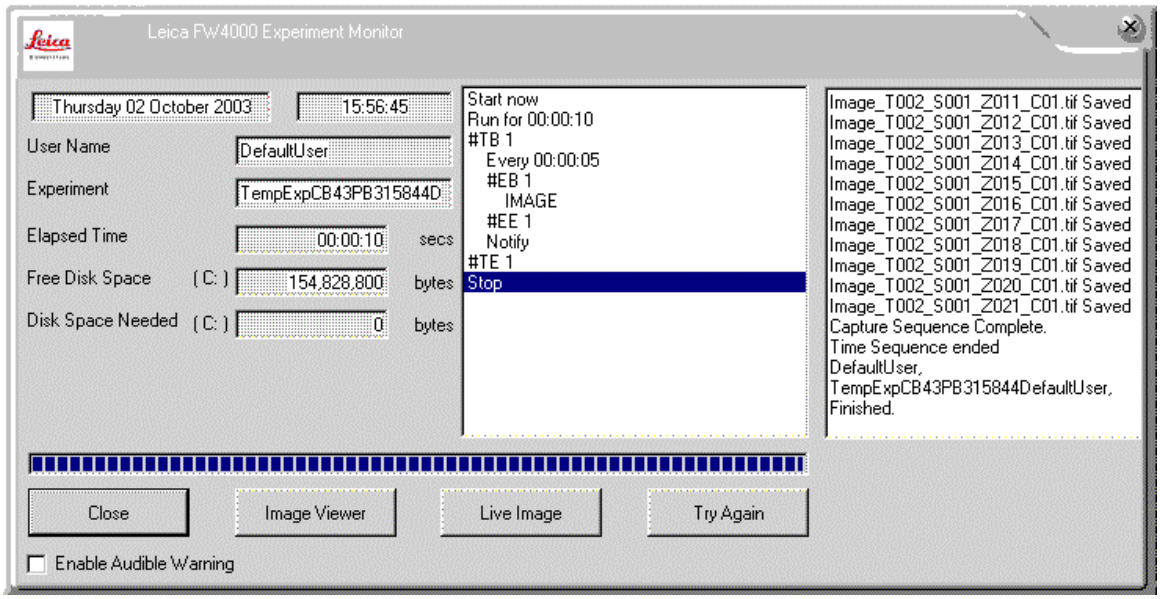
- Run the image capture program automatically on entering this element of the end-to-end process.
- Enable monitoring.
- Stop or pause capturing images to modify the sequence, or view the images captured so far into the sequence.
- Rerun the time sequencing program if the set of results is deficient in some way.

➡ To start capturing images, click the **Capture** button in the Environmental Setup process flow.

The **Image Capturing** dialog appears. When the dialog first appears, there is a short time interval before the time sequencing runs. When the time sequencing runs, you can see progress of the run in the right window.



When the time sequence program is complete, you will see an appropriate message at the end of the run details, and the **Stop** button will become a **Close** button. See the end of time sequence run dialog:



➡ To launch the Image Viewer directly from the Capture Image dialog, click **Image Viewer**.

Note: You might want to view the captured images after each time sequence to ensure that you have captured the images you want.

Note: This way of working provides a hands-off, automated image capture sequence (such as time sequence or Z-stack image sequences) method.

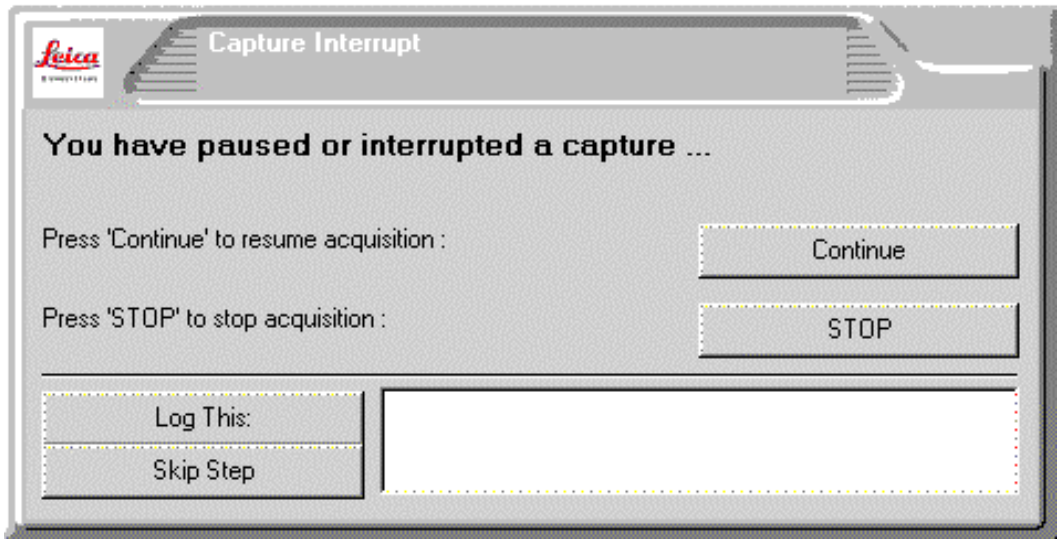
- Alternatively, set the image frame and exposure you require, whilst the image is **Live**, and click **Stop.** Click the **Image Viewer** button to display the Image Viewer where you will see the captured image displayed. Adjust the size and screen position of the Image Viewer so the image viewer and XY dialog are open alongside each other.

- To exit from this stage in the Experimental Process, click the **Close** button in the bottom left corner of the **Capture Images** dialog.

Using the Image Capturing Tools

To stop or pause capturing images in flight:

1. Click **Stop** to display the **Capture Interrupt** dialog.



2. Click **Continue** to continue the time sequence run, or **STOP** to stop the image capture acquisition.
3. If required, add a note in the **Log This** text box.
4. If required, click **Skip Step** to move the experiment forward to the next time sequence.

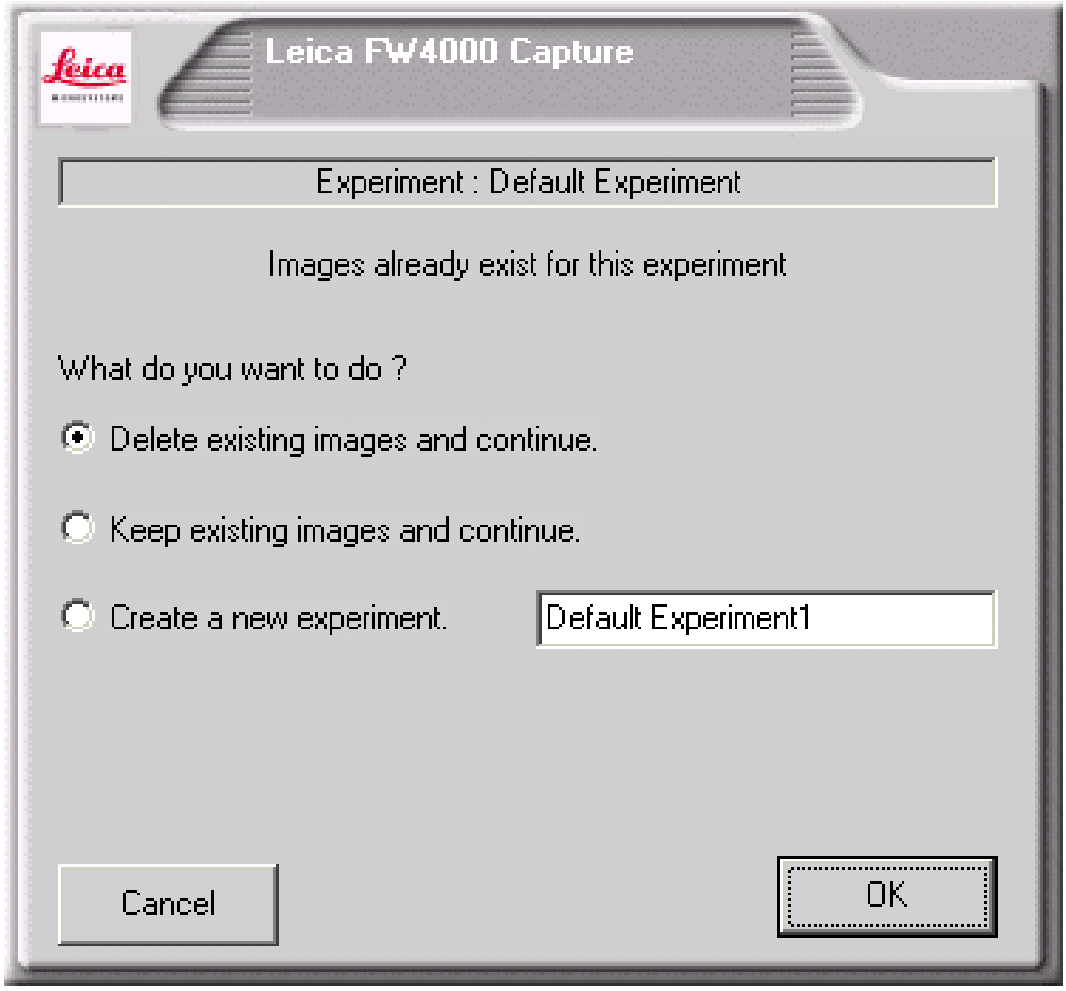
➡ Click **Close** to close the image capture dialog.

➡ Click **Try Again** to rerun the time sequence.

Note: When you rerun the time sequence, complex time sequence programs capture large numbers of images and thus require high values of filestore to accommodate those captured images.

➡ To view the images you have captured, click Image Viewer.

When you click Capture again, you see the **FW4000 Capture** dialog. Check the box appropriate to the action desire, and then click **OK**.



Refer to: *Chapter 9, Enhancing your Image* for a detailed description of how to view the images and manipulate them to maximise your analysis of the results.

Refer to: *Chapter 2, Setting General Preferences* for a description on how to set which image is shown in the Image Viewer.

Chapter Six: Reviewing Images

Chapter Overview

This chapter contains the following topics:

- Introduction
- Viewing Images
- Displaying and Selecting Images
- Displaying and Comparing Sequential Images
- Previewing Composite Images
- Building a Profile for the Images to be Selected
- Saving Selected Images
- Defining the Image Gallery Settings
- Preparing Selected Images for Processing
- Preparing Selected Images for Publishing
- Replacing Images

Introduction

This chapter describes how to view the images captured during the image capture stage of the Environmental Setup process flow. This review only allows you to view the images from your currently loaded experiment, or from the experiment you have just run.

However, in the Lab Book, you can view the images from a chosen experiment in the gallery, by selecting an experiment and clicking the **Gallery** button.

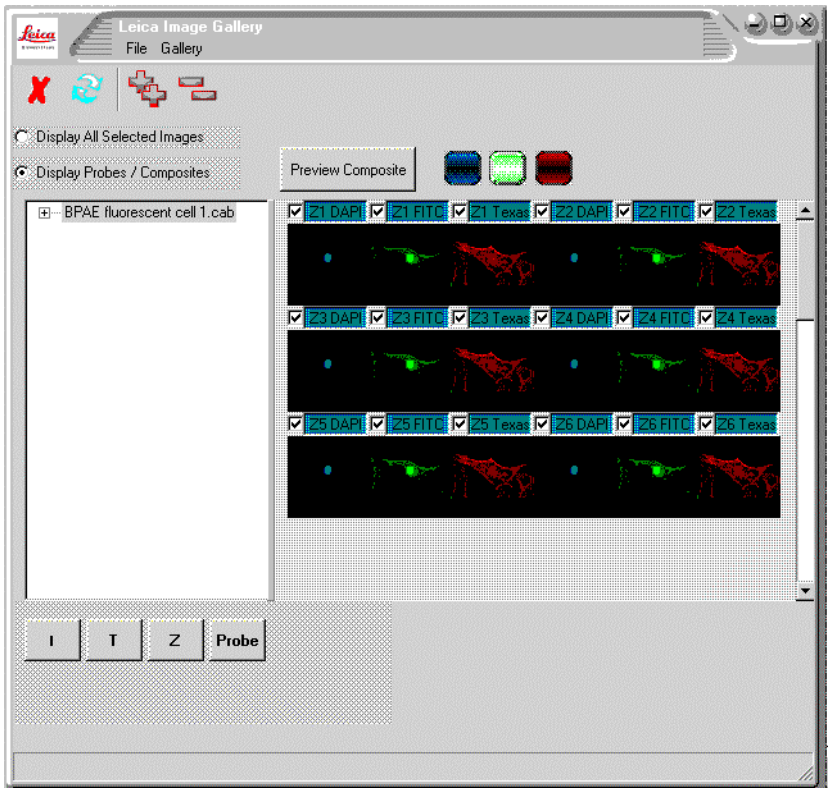
Refer to: *Chapter 7, Processing Images* for information on measuring, enhancing and deconvolving your images.

The software allows you to view images in two distinct ways:

- Display the images in the sequence in which they were captured (Z or T) or by fluorochrome (probe).
- Display the images as a composite image of the output of all probes (I, N, T and Z) but based on one that you select. The images can be manipulated to produce a composite image by combining the effects of different probes.

Viewing Images

➡ To review the images you have captured, click **Review** in the Environmental Setup process flow. The **Leica Image Gallery** dialog appears.



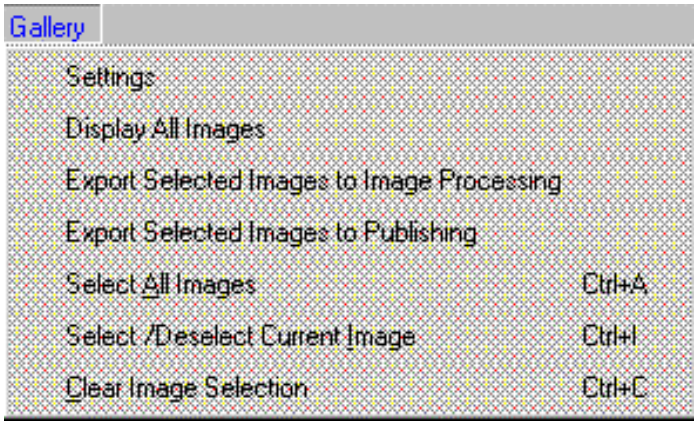
➡ When you select **Display Probes/Composites** and reorder the probes sequence, the Reorder button appears. When you click **Reorder**, the radio button **Display All Images** is selected automatically.

➡ To exit from this stage in the Environmental Setup process flow, click ⊗ in the top right corner of the **Leica Image Gallery** dialog.

Tip: You can automatically launch the gallery with the images for your experiment displayed after the experiment has finished capturing. This option is available by opening the FILE menu and choosing the General tab from Preferences.

Displaying and Selecting Images

The image Gallery menu and the image radio buttons allow you to display and select all the images you have captured, or only a subset of them.

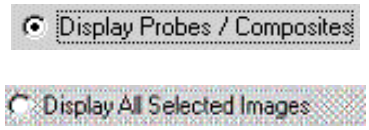


- To display all the captured images, click the image gallery **Gallery** menu and click **Display All images**.
- To select all the captured images, click the image gallery **Gallery** menu and click **Select All Images**, or press the **Ctrl+A** keyboard keys, or click the select all images icon (see the icon to the right). All displayed images will have **Select** checked.

- To clear the selection of all the captured images, click the image gallery **Gallery** menu and click **Clear image selection**, or press the **Ctrl+C** keyboard keys, or click the clear images icon (see the icon to the right). All displayed images will have **Select** unchecked.

- To select or deselect the current image, click the image gallery **Gallery** menu and select an image. Click **Select/Deselect Current Image**, or press the **Ctrl+I** keyboard keys.
- To delete selected images, click the image gallery **Gallery** menu and select the images to be deleted. Click **Delete Selected Images**, or press the **Ctrl+D** keyboard keys, or click the Delete icon. Click Yes or No in the Delete Conformation dialog that is displayed.

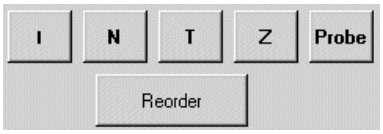
➡ To display images for only one probe, select the **Display Probes/Composites** radio button and select the desired probe. To show images for all the probes, select the **Display All Selected Images** radio button.



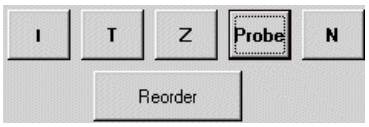
Displaying and Comparing Sequential Images

The images viewed here are the same images that you captured earlier. They have not been manipulated in any way to produce a composite image.

To reorder the image display sequence:



1. Click the left mouse button to select one of five experimental parameters (an arrow is superimposed upon the parameter you want to move).
2. Move the parameter buttons to the sequence you want by dragging and dropping the selected buttons to left or right individually.
3. When you have moved the buttons to the order you want, click **Reorder** to confirm the selected order.



4. The image display order changes to match the reordering of the parameters.
5. Repeat steps 1 to 3 to compare different images until you are satisfied that you have a suitable set.

To display only the images in the selected treeview node, click the images in the left **Lab book View** and select combinations of probe, as you require.

Note: If the captured images are unsuitable then start again by reviewing the environmental parameters and possibly the time sequencing.

Note: The parameter Reorder button appears only when you change the parameter order.

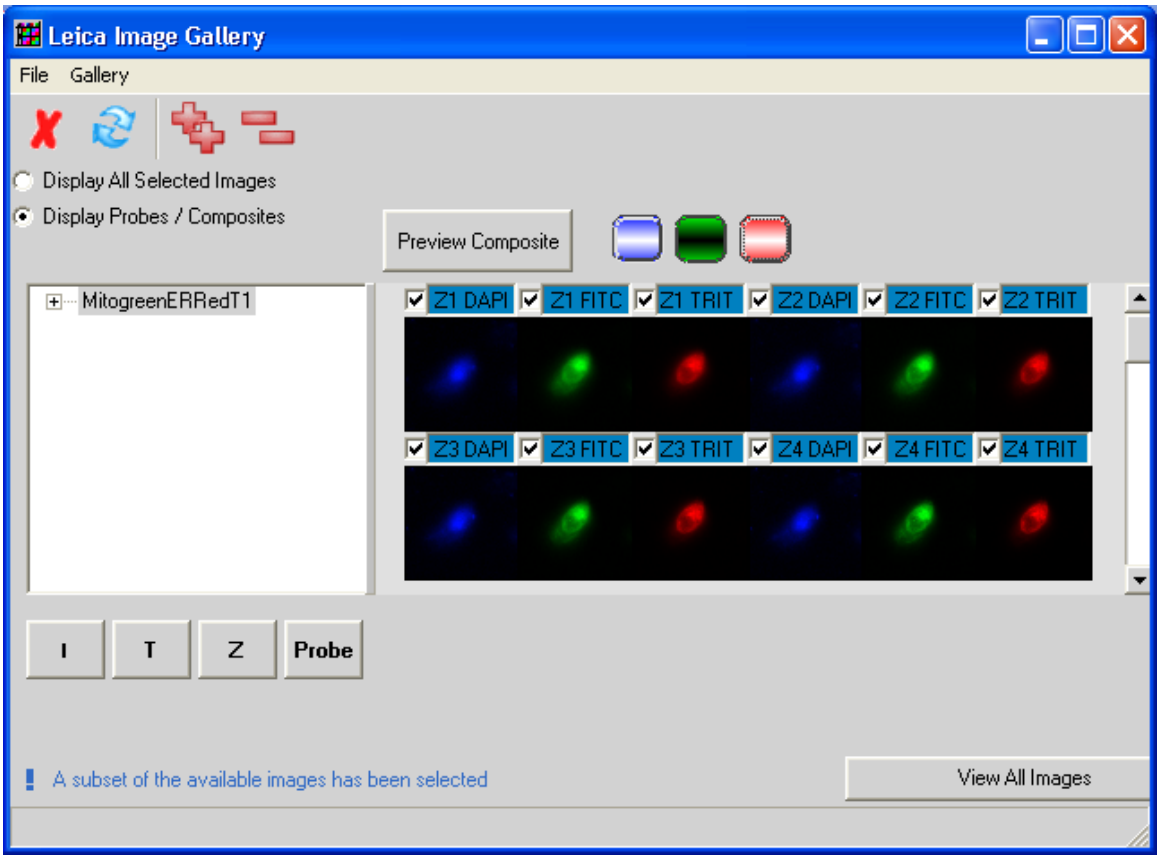
Note: Once you have defined an image set to view and process, you can change the display order of the images in the gallery. To reorder the images, resort the images, by changing the sequence position of the **I** button.

Previewing Composite Images

The composite images viewed here have been produced by mixing output from a selection of the probes. The composite images allow you to identify more clearly activities occurring in your experiment samples.

To develop composite images:

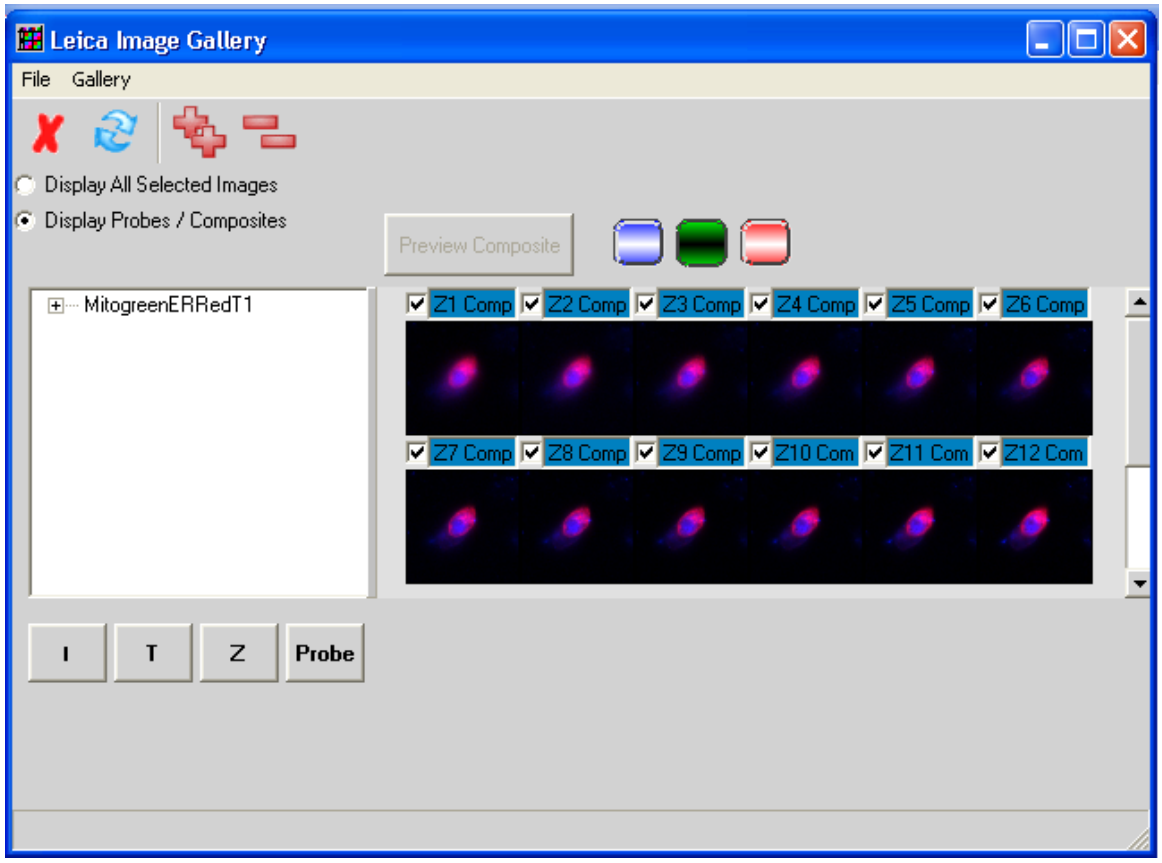
1. Move the **Probe** button so that it is positioned at the right end of the four buttons (the exact number depends on whether stage positioning, zstack and time sequence are being used), and click **Reorder**.
2. Click on the **Display Probes / Composites** radio button.



3. Click the required **Probe**(s) to use for producing composite images.
4. Click **Preview Composite**.

5. Repeat steps 3 and 4 as you view a variety of composite images.

Note: This allows you to quickly preview composite images of different combinations of probes, but only produces thumbnail images. When you are happy with the combination of probes, use the Image Viewer to define the precise composition of the composite images and to create the full size images.



Building a Profile for the Images to be Selected

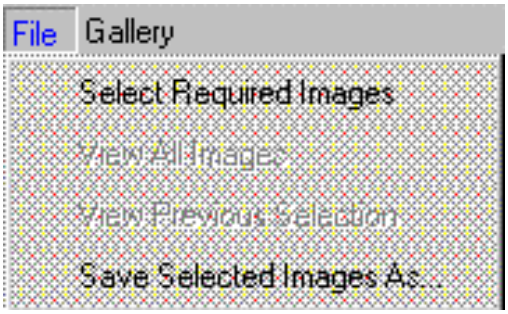
The **Select Required Images** dialog allows you to select particular images to review, from the complete set you captured, by defining the precise criteria that **Leica FW4000** uses to find those images. The dialog allows you to build a profile of the setup criteria for the images to be selected, and match the captured images from the image gallery with that profile.

As you select different parameters to build the profile for the image you want to select, the information displayed in the Filter area of the **Select Required Images** dialog changes.

After setting up each of the filter criteria, you build up the total filter profile by clicking **Insert**. The profile appears in the field in the centre of the filter area.

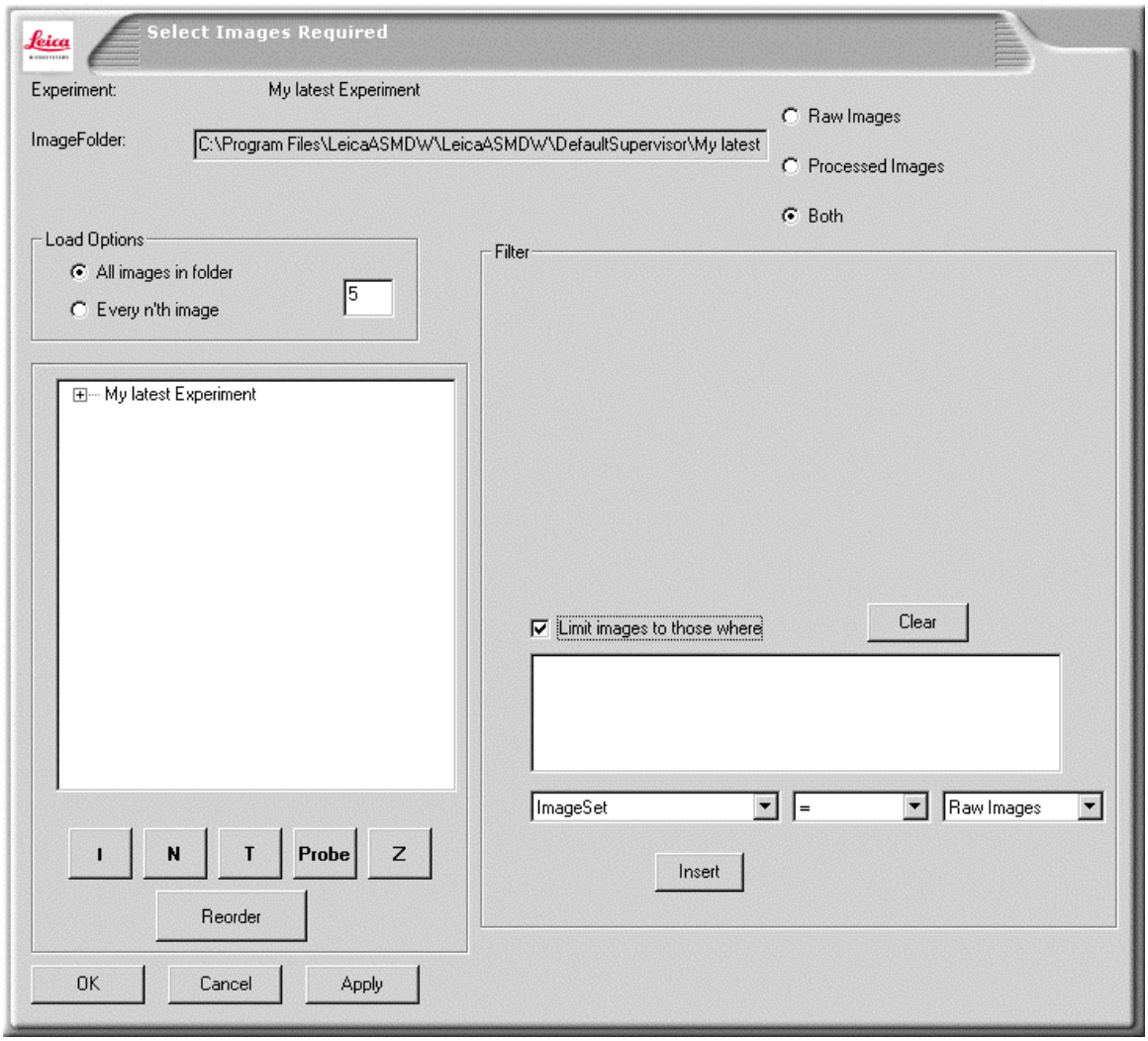
To build the image profile for images you want to process:

1. Click the Image Gallery **File** menu, and then click **Select Required Images** to display the **Select Required Images** dialog.



Note: When you have an active image selected from the **Select Required Images** dialog, you are returned to display all images. Then you can toggle between a view of all images and the previous selection.

Note: In this mode, a context sensitive button appears at the bottom of the dialog to enable more effective toggling between the two image views.



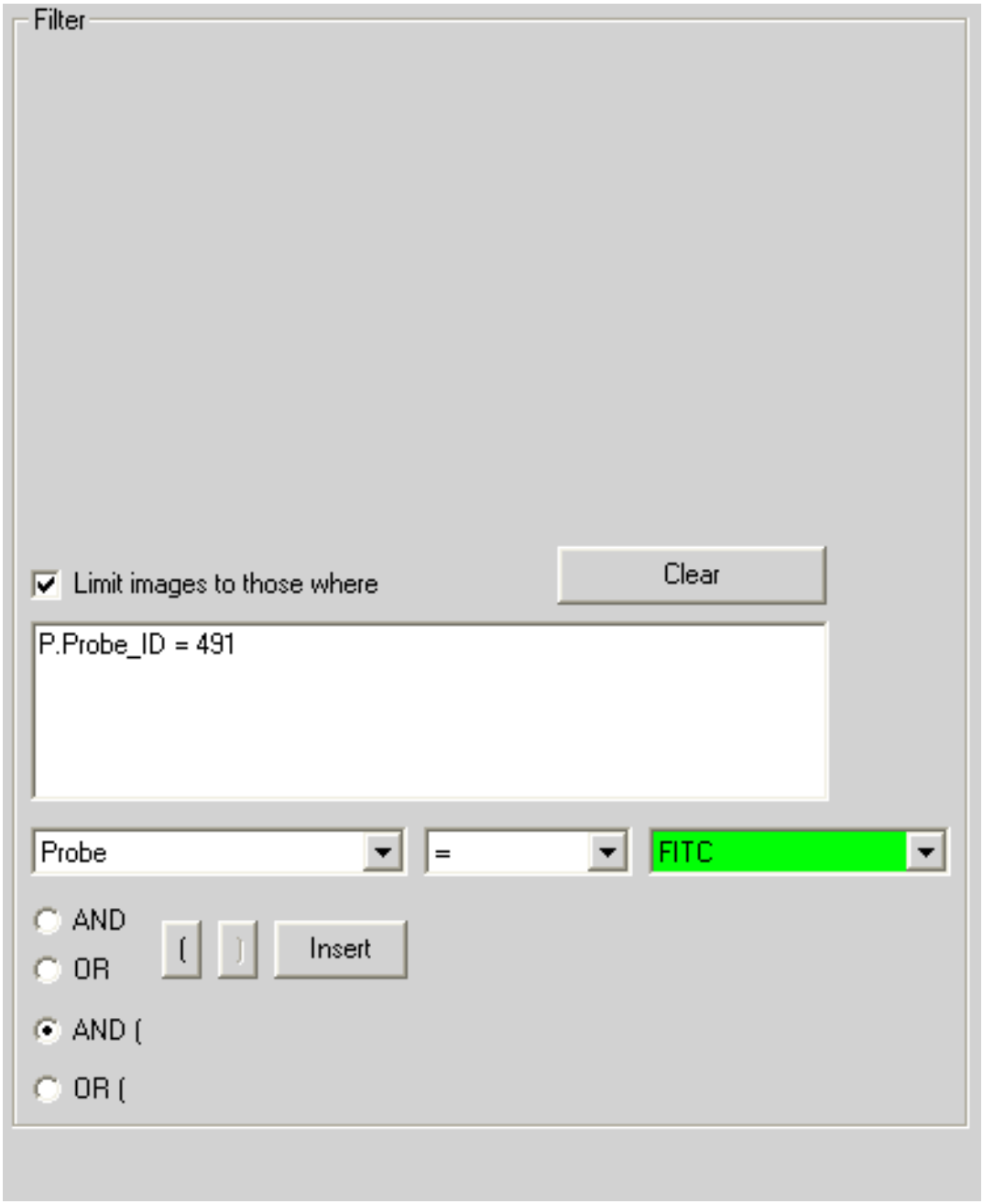
2. Click the appropriate **Raw Images** or **Processed Images** radio button.
3. Define the **Load Options** by clicking the appropriate radio button and typing the required number of images.

4. Define the experiment parameter order you want to use.

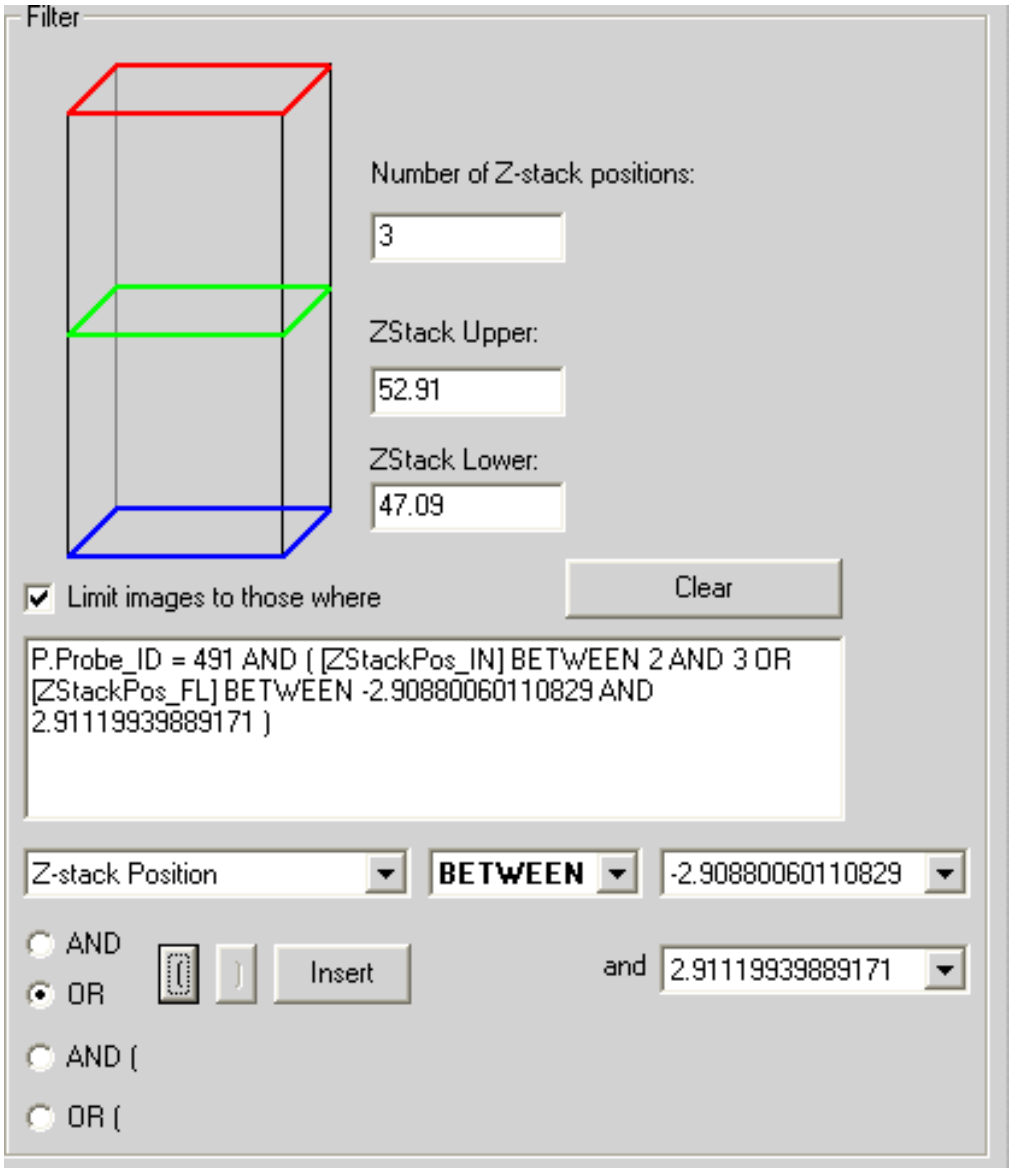
Refer to: *Chapter 6, Displaying and Comparing Sequential Images*, for a description on reordering the parameters.

5. Define the filter required by building up a filter profile. Use the **Filter** dialog.
6. Check the **Limit images to those where** box.

7. Select a filter by clicking **Probe** in the left filter window. Highlight the required filter from the right menu.
8. Click **Insert**.

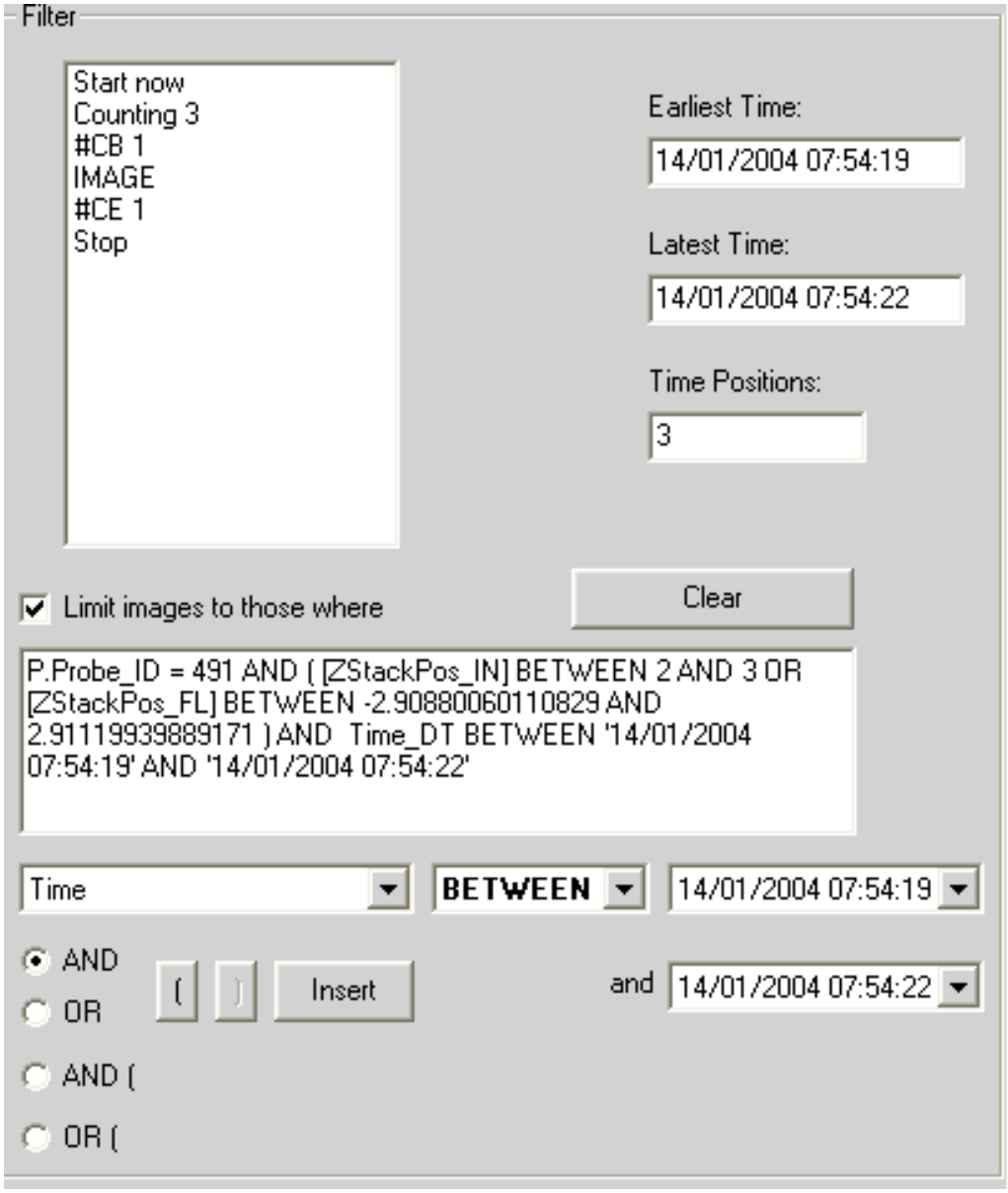


9. Select **Z-stack Position Number** in the left filter window, in the following figure.
10. Select the function **BETWEEN,** from the centre window.
11. Type values for upper and lower limits in the two fields provided.
12. Click one of the **AND** or OR radio buttons.
13. Click **Insert**.

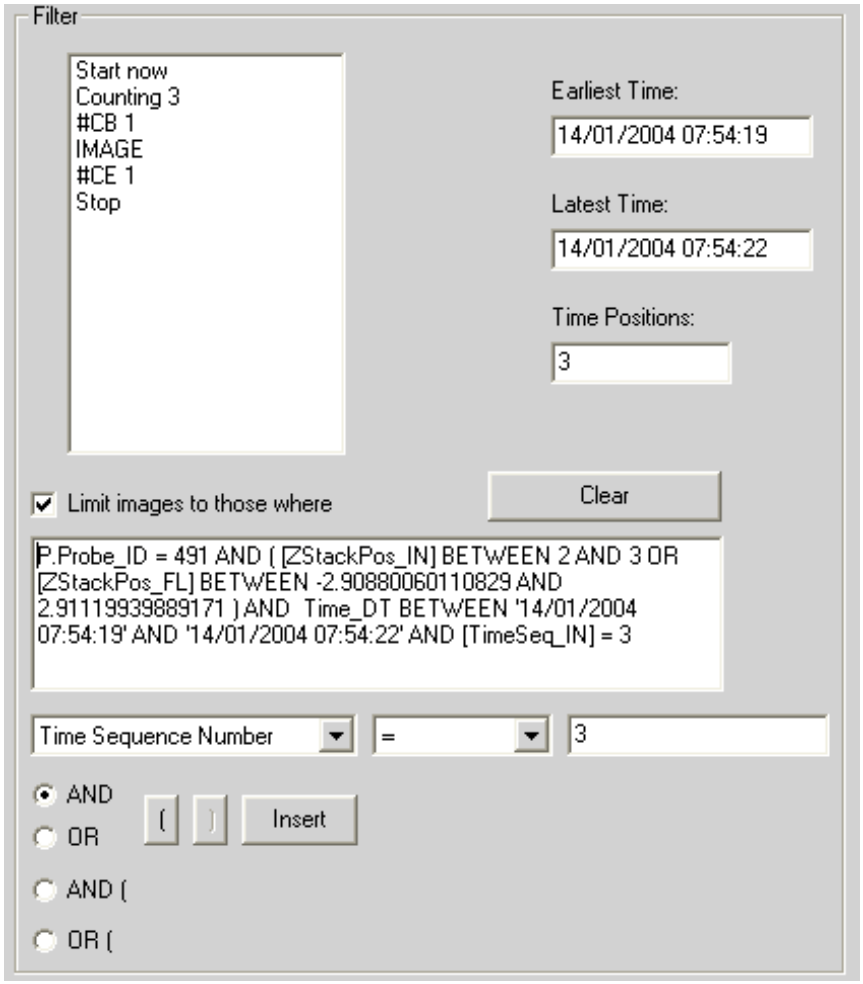


14. Select **Z-stack Position** in the left filter window.
15. Click one of the **AND** or **OR** radio buttons.
16. Click **Insert**.
17. If necessary, click a closing bracket to match the opening bracket in the previous AND(or OR (.

18. Select **Time** in the left filter window.
19. Click one of the **AND** or **OR** radio buttons.
20. Click **Insert**.



21. Select **Time Sequence Number** in the left filter window.
22. Type the required time sequence number in the right field.
23. Click one of the **AND** or **OR** radio buttons.
24. Click **Insert**.



Note: If you use a combination of AND and OR in your selection, you must insert opening and closing brackets to make the meaning unambiguous. Otherwise, the selection will not be what you intended.

Note: The function you select changes the range of information you must supply in the right window.

25. Click **Apply** to apply the image profile without closing the dialog. If you are dissatisfied with the new profile, click **Clear** and repeat steps 7 to 23 until you are satisfied.

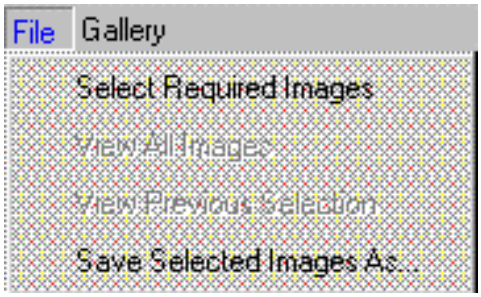
26. Click **OK** to accept the total image profiles, or click **Cancel** to cancel the operation.

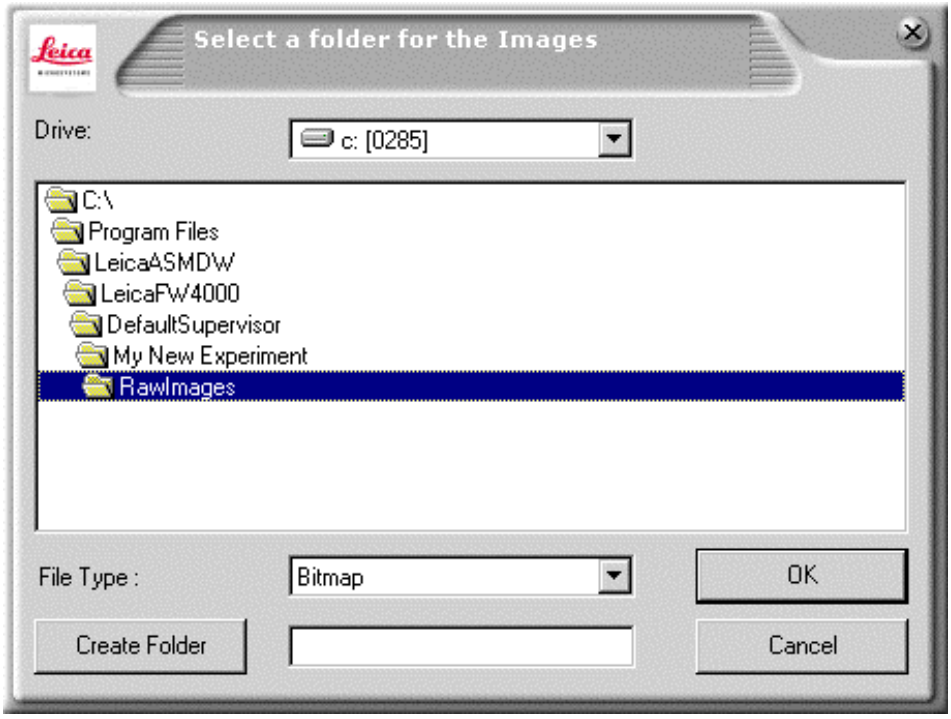
Saving Selected Images

When you have selected the particular images to review, from the complete set you captured, you may save them.

To save the selected images you want to process:

1. Click the Image Gallery **File** menu, and then click **Save Select Images As** to display the **Select a folder for the Images** dialog.



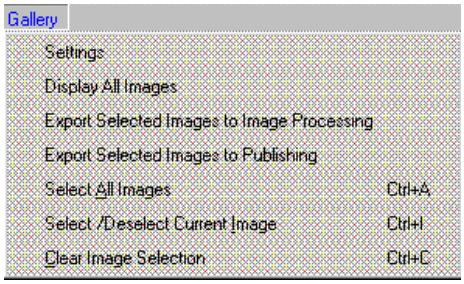


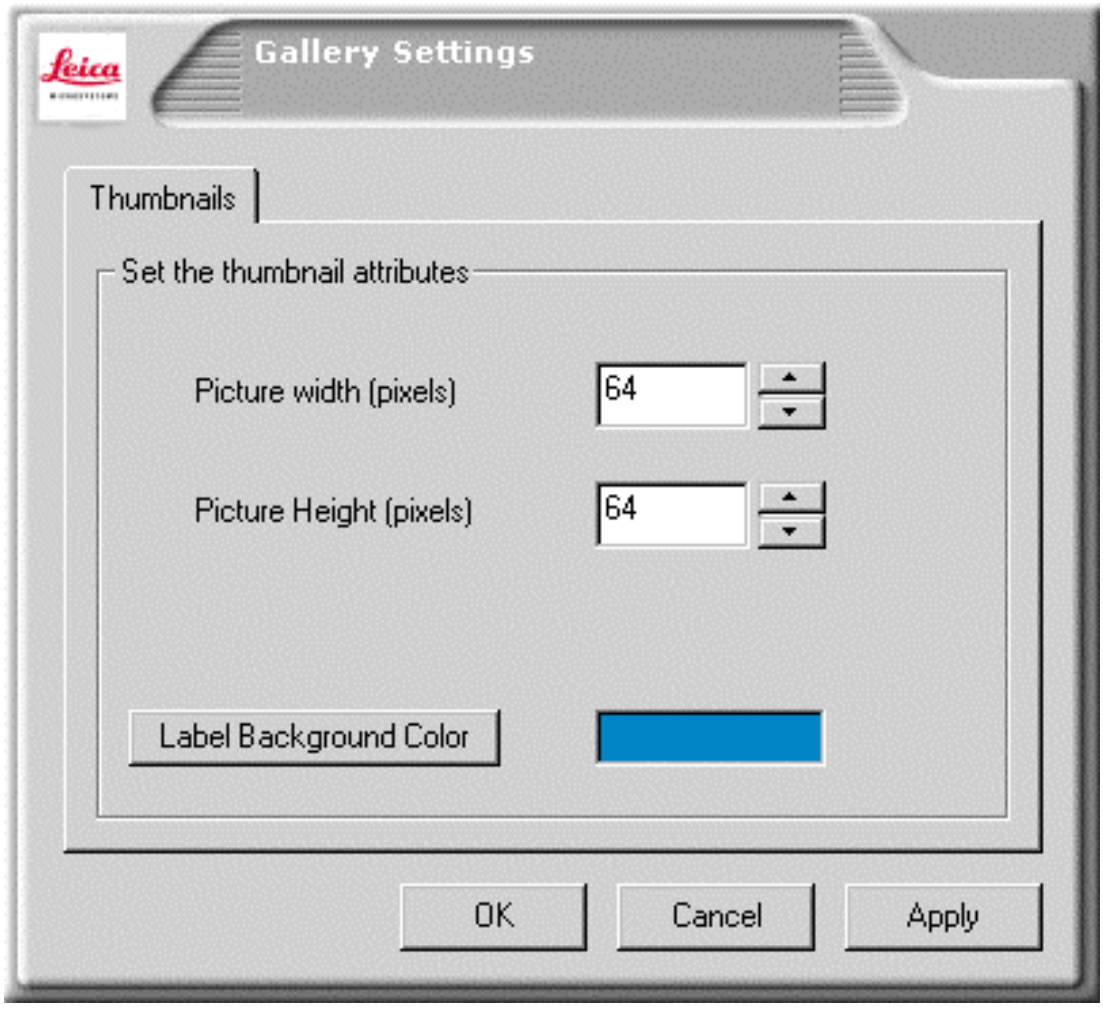
2. Select the file type from a choice of Bitmap, JPEG or TIFF.
3. Create a new folder, or browse to an existing folder, as appropriate.
4. Click **OK** to save the images, or click **Cancel** to cancel the operation.

Defining the Image Gallery Settings

To define the look and feel parameters for the Image Gallery:

1. Click the image gallery **Gallery** menu and click **Settings** to display the **Gallery Settings** dialog, which is superimposed on top of the **Leica Image Gallery**.





2. Redefine **Picture width** and **Picture Height** by using the up and down arrows.

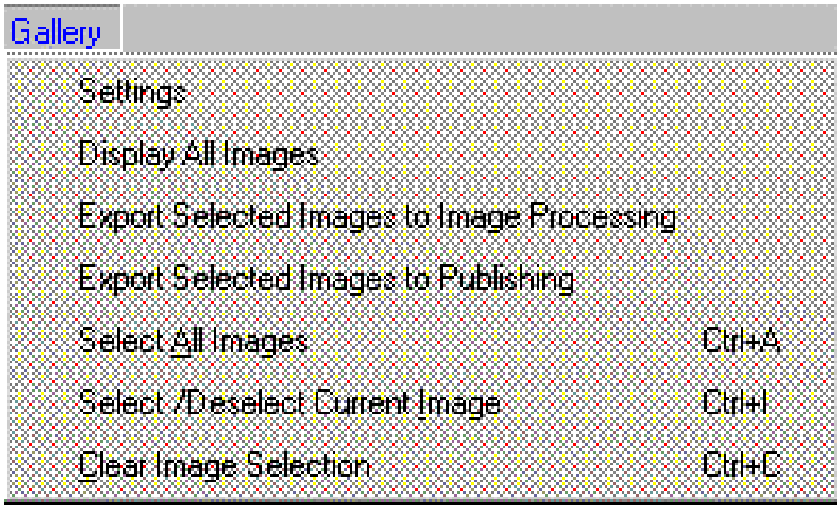
3. Redefine the Label Background Colour, as required, by clicking the **Label Background Colour** box, choosing the required colour from the **colour palette**, and clicking **OK**.
   The **Gallery Settings** dialog now appears again, with the new settings defined.
4. Click **Apply**. The Image Gallery, viewed behind the gallery settings dialog, changes to reflect the newly defined attributes.
5. If you are dissatisfied with the effect, repeat steps 2 to 4 until you are satisfied with the **Leica Image Gallery** attributes.
6. Click **OK** to accept the new attributes, or click **Cancel** to cancel the operation.

Preparing Selected Images for Processing

To process your images in the Image Viewer, double-click on one of the selected images in the gallery. If you want to process the images in **Leica Deblur and 3D Visualise,** if you have purchased that option, you must specifically select them and export them to Image processing.

To prepare images for processing:

1. Open the Leica Image Gallery.
2. Select the individual images you want to process.
3. Click the Image Gallery **Gallery** menu and click **Export Selected Images to Image Processing**.
4. Close the **Leica Image Gallery**.



➡ Alternatively, to open the image viewer you can double-click the required image in the gallery.

Preparing Selected Images for Publishing

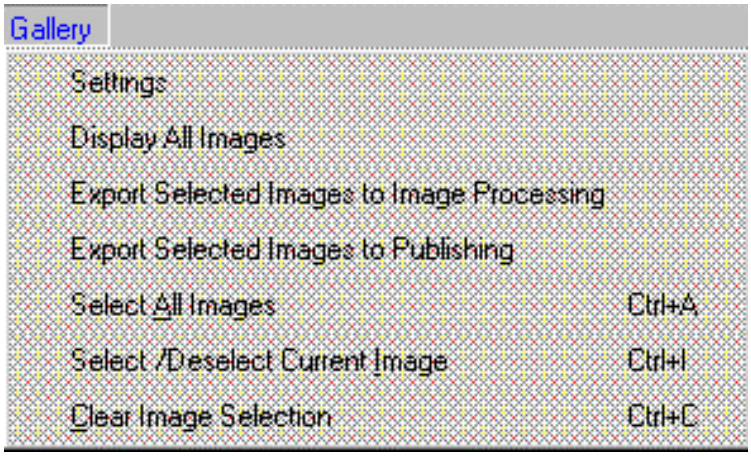
Before you can publish your images for a montage report, you must specifically select them and export them to the publishing area.

Note: If you just want a report showing a composite image and its component probe images, you can generate this by selecting the Publish icon without going through the gallery.

Note: Before you export a set of images for publishing, ensure that you have selected a sensible set of images.

To prepare images for publishing:

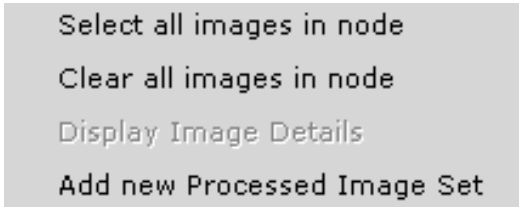
1. Open the **Leica Image Gallery**.
2. Select the individual images you want to publish.
3. Click the Image Gallery **Gallery** menu and click **Export Selected Images to Publishing**.
4. **Close** the **Leica Image Gallery**.



➡ To select or clear images for processing and publishing, right-mouse click a branch of the Lab Book view to show the context sensitive menu.

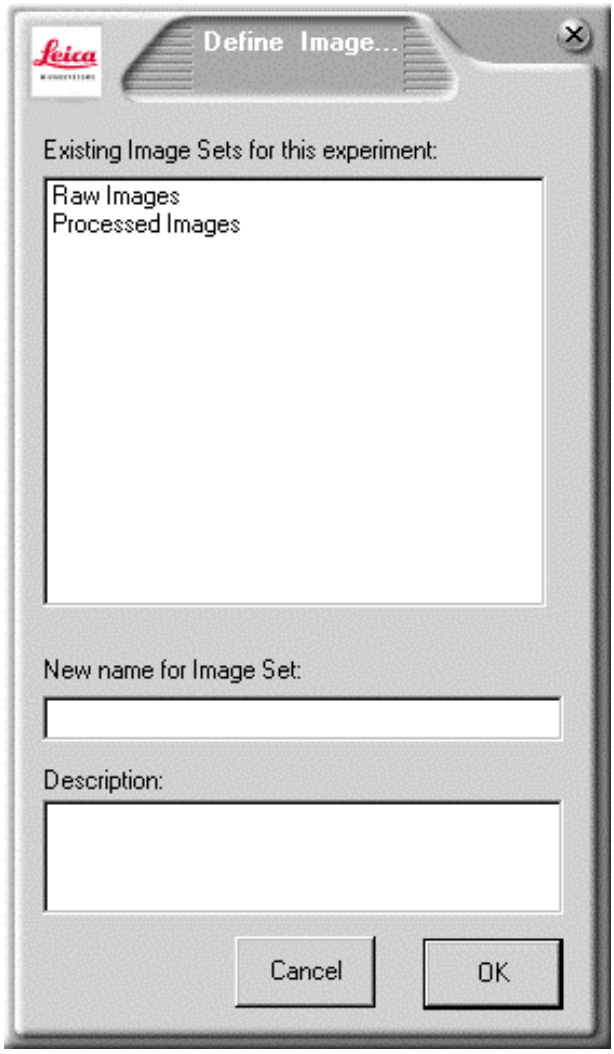
➡ To add a new image set:

1. Right-click on the Experiment node at the top of the tree view.
2. Click **Add new Processed Image Set** and enter the name of the image set in the **Define Image** Set dialog.
3. Click **OK**.



➡ **To change the name of an image set**:

1. Right-click on the image set name.
2. Click **Rename Image Set** and enter a new name for the image set in the **Define Image** Set dialog.
3. Click **OK**.



Note: This action allows you to change subsets of processed images and you can enter into a defined image set within the Lab Book. (The Lab Book offers you several ways of entering images: raw, processed and subsets.)

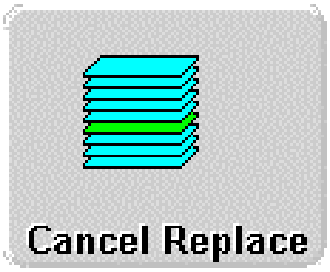
Replacing Images

When you review images in the gallery and need to replace one, recapture that image using the image capture tools.

To replace an image, or images, in the image viewer:

1. Either:
   - Select the image to be replaced in the Image Gallery.
   - If you are replacing more than one image, select the node in the tree view containing the images to be replaced.
2. Click the **Replace Images** icon, to open the Live Image dialog.

Note: The status icon on the desktop shows the animated **Cancel Replace** icon.



3. Select the image, to be replaced, in the Live Image dialog.
4. Capture the image. To capture images at several different stage positions, use the Capture icon on the main form.

Refer to: *Chapter 4, Defining the Live Acquisition* for a detailed description of capturing a single image.

5. If you decide not to replace the images after all, click the **Cancel Replace** status icon on the desktop.

6 To replace the images, press the Review icon and then select the Accept Replacement Images icon in the gallery. Alternatively, you can reject the replacement images, or add them to the experiment in addition to the original images.

Chapter Seven: Processing Images

Chapter Overview

This chapter contains the following topics:

- Fundamental Principles of Image Processing
- Starting the Process Environment
- Image Toolbar
  -
  - Z-stack Playback Toolbar
  - Time Sequence Playback Toolbar
  - Customising Your Image Toolbar
- Using the Processed Image Set
- Windowing tools
  - Erasing
  - Send Images to Leica QWin
- No Neighbours and Nearest Neighbours Deconvolution
- Defining a Region of Interest
  - Applying an Overlay

Fundamental Principles of Image Processing

When you acquire images with **Leica FW4000**, the brightness and contrast of the image are affected by various factors. These factors include:

- Camera integration time
- Video levels
- Camera gain and offset

The sharpness and resolution are dictated largely by the quality of the optical microscope and camera that you use to acquire the image. **Leica FW4000** provides many facilities to help you acquire high-quality images.

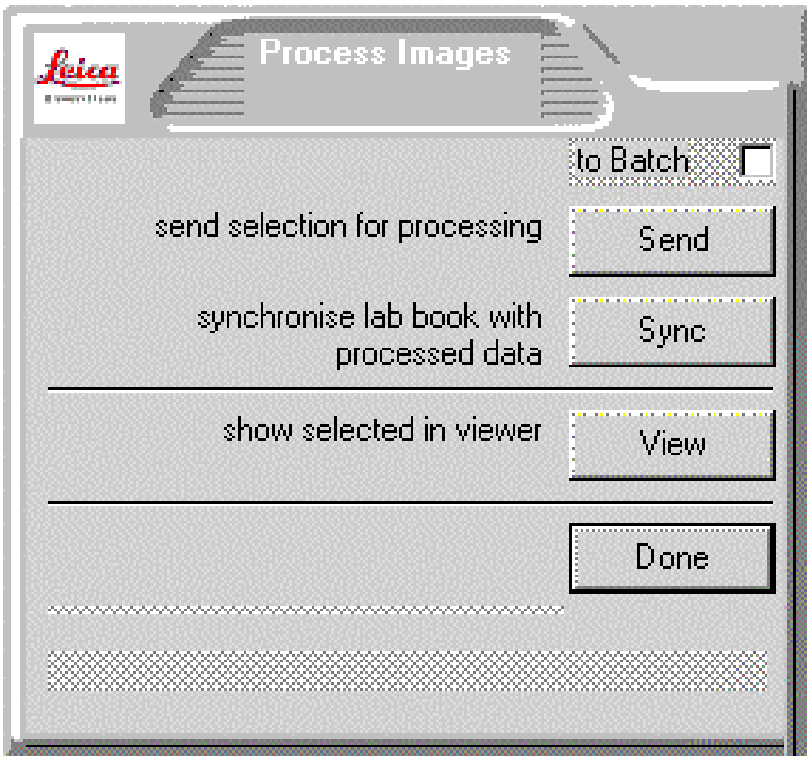
Certain image processing techniques are available to allow you to enhance images after they have been captured. These include the ability to:

- Highlight features of interest by eliminating background fluorescence.
- Adjust image brightness and contrast to improve visualisation of detail.
- Reduce background fluorescence intensity.
- Increase signal intensity.
- Automatically optimise the image contrast range.
- Increase the sharpness of details and edges.
- Remove unwanted debris and artefacts.
- Correct for image misalignment using shifting.

These and other image processing options provide flexibility in further enhancing the appearance of images, even after you have captured and stored them.

Starting the Process Environment

➡ To process images click **Process** in the experimental setup. The **Process Images** dialog is displayed.



➡ Click **View** in the **Process Images** dialog to open the **Image Viewer**.

Note: Before you can process your images in the **Image Viewer** you must already have specifically selected them and exported them for image processing.

Refer to: *Chapter 6, Preparing Selected Images for Processing*.

Note: This dialog is only seen if you have purchased a licence to run **Leica Deblur and 3D Visualise**. If you have not purchased this licence, the **Image Viewer** is automatically launched.

Leica Image Viewer

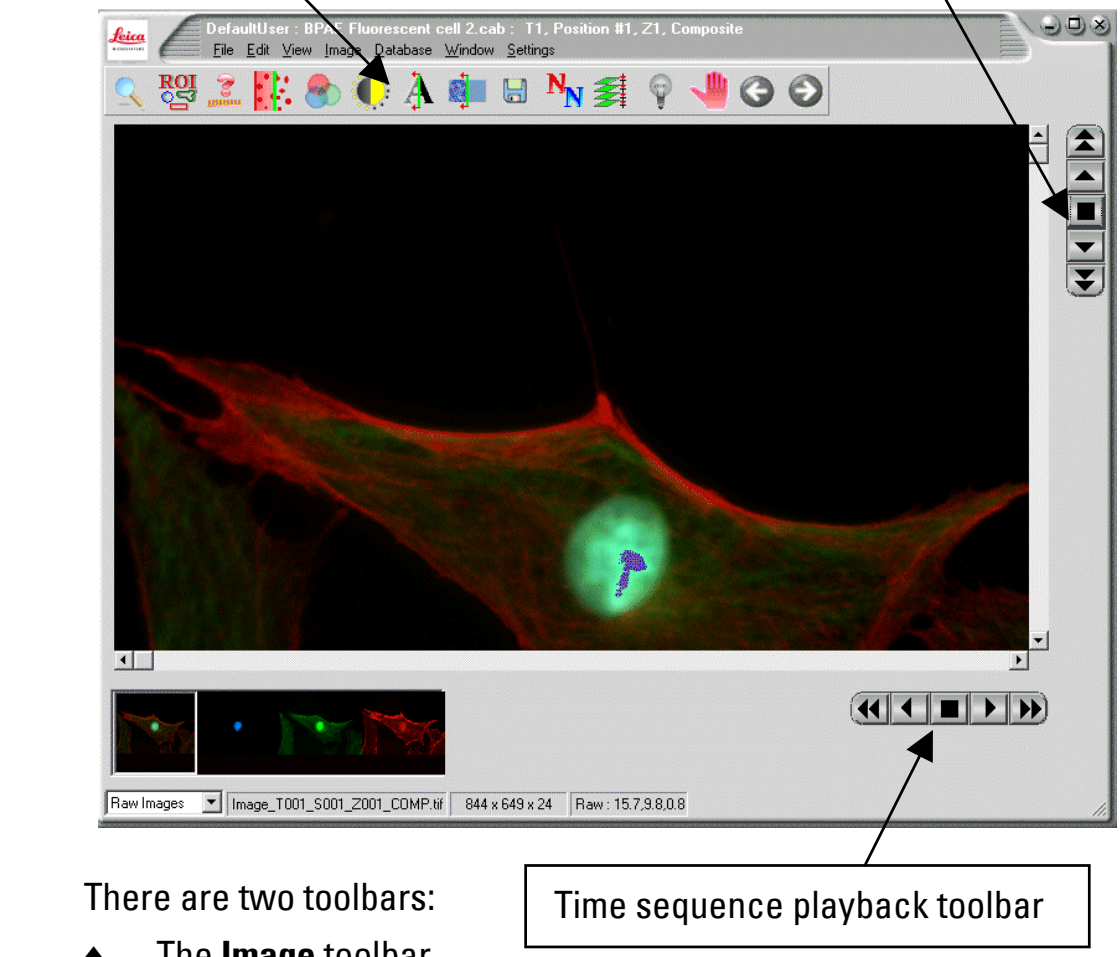


Image toolbar
Z-stack playback toolbar
DefaultUser : BPAE Fluorescent cell 2.cab : T1, Position #1, Z1, Composite
File Edit View Image Database Window Settings
ROI
Raw Images
Image_T001_S001_Z001_COMP.tif
844 x 649 x 24
Raw : 15.7,9.8,0.8
Time sequence playback toolbar

There are two toolbars:

- The **Image** toolbar.
- The **Z-playback** toolbar.
- The **Time sequence playback** toolbar.

Image Toolbar

The **Image** toolbar allows you to perform a variety of operations to enhance the image.

Select allows you to draw a selection box on the image by holding down the left mouse button and dragging. This box can subsequently be copied to the clipboard or moved to the **Print** dialog.

Zoom allows you to zoom in and out of the image. Left-clicking zooms in, and right-clicking zooms out.

Erase erases the defined area. Define an area by holding down the left mouse button and drawing around the required area. To remove all parts of the image except the defined area, draw around the area with the right mouse button held down.

Define Regions allows you to define a region of interest (ROI) for the fluorochrome currently displayed using the current ROI settings. The defined region can be used for image enhancement by selecting **Use ROI** from the Image menu. The **Select Regions of Interest** dialog will appear.

Refer to: *Chapter 8, Performing Measurements,* for information on the Select Regions of Interest dialog.

Annotation opens the **Annotation Toolbox**, and allows you to annotate an image with various items including text, scale bars, shapes and straight-line distances.

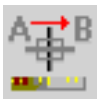
Annotation may be saved with the image or to a specified file. This is useful in defining an annotation template, which can be used with many images.

Refer to: *Chapter 8, Annotating your Image,* for information on annotation.

Calibrate allows you to calibrate the system. Using a stage micrometer and a live image, draw a line corresponding to a known distance on the micrometer. Click this button to display the **Calibration**.

Refer to: *Chapter 4, Setting up an Environment for Experiments* for more information on calibrating **from the Live Image Setup dialog**.

Refer to: *Chapter 8, Calibrating an Image* for more information on calibration.



Measure Distance opens the **Measurements** dialog, and allows you to perform various measurements on the image.

Refer to: *Chapter 8, Performing Measurements,* for more information on measuring distance.

Remove Background removes the background from the image. The **Remove Background** dialog allows you to adjust the threshold and specify the display colour. Click and hold down the **toggle** button to compare the image before and after removal of the background.

Refer to: *Chapter 9, Removing the Background* for more information on removing the background.

Make a **composite** fluoro image.

Adjust Contrast allows you to adjust the gamma, contrast and brightness of an image using the **Image Contrast** dialog.

Measure Intensity allows you to measure the intensity of the probes at different points in the image.

Refer to: *Chapter 8, Measuring Probe Intensity f*or more information on measuring intensity.

Sharpen allows you to sharpen the image.

Smooth allows you to perform noise reduction on the image.

Save the image you are processing currently. This tool enables you to save an image through a number of processing stages. Thus, the sequence of processed images can be included in reports and published documents.

Deconvolution removes the out of focus information from the image using a 2D deconvolution algorithm.

Max Projection is a single image from the Z-stack that uses the maximum intensity pixels from the entire stack.

Brightfield inverts the image, making dark pixels light and pseudocolours the image to black and white.

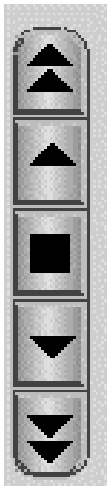
Pan allows you to move the image around in the image window.

Previous Stage Position moves to the previous stage position (if any)

Next Stage Position moves to the next stage position (if any)

Z-stack Playback Toolbar

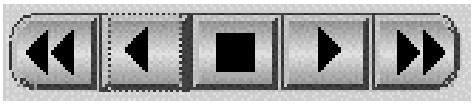
The **Z-stack playback** toolbar allows you to play, reverse and step through the images in the Z-stack.



- To run up through the images in the Z-stack, click the **Run Upwards** button.
- To step upwards through the Z-stack, one image at a time, click the **Step Upwards** button.
- To stop the playback of the Z-stack display, click on the **Stop** button.
- To step downwards through the Z-stack, one image at a time, click the **Step Downwards** button.
- To run down through the images in the Z-stack, click the **Run Downwards** button.

Time Sequence Playback Toolbar

The **Time sequence playback** toolbar allows you to play, reverse and step through the images in the time sequence.



➡ To run up through the images in the **Time Sequence**, click the **Run Backwards** button.

➡ To step backwards, one image at a time, click the **Step Back** button.

➡ To stop the playback of the **Time Sequence**, click the **Stop** button.

➡ To step forwards, one image at a time, click the **Step Forward** button.

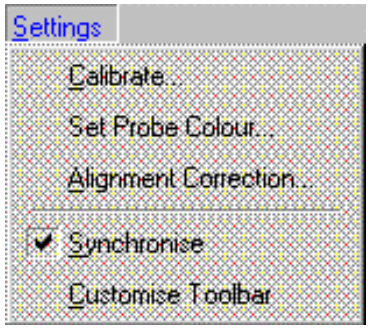
➡ To run down through the images in the **Time Sequence**, click the **Run Forwards** button.

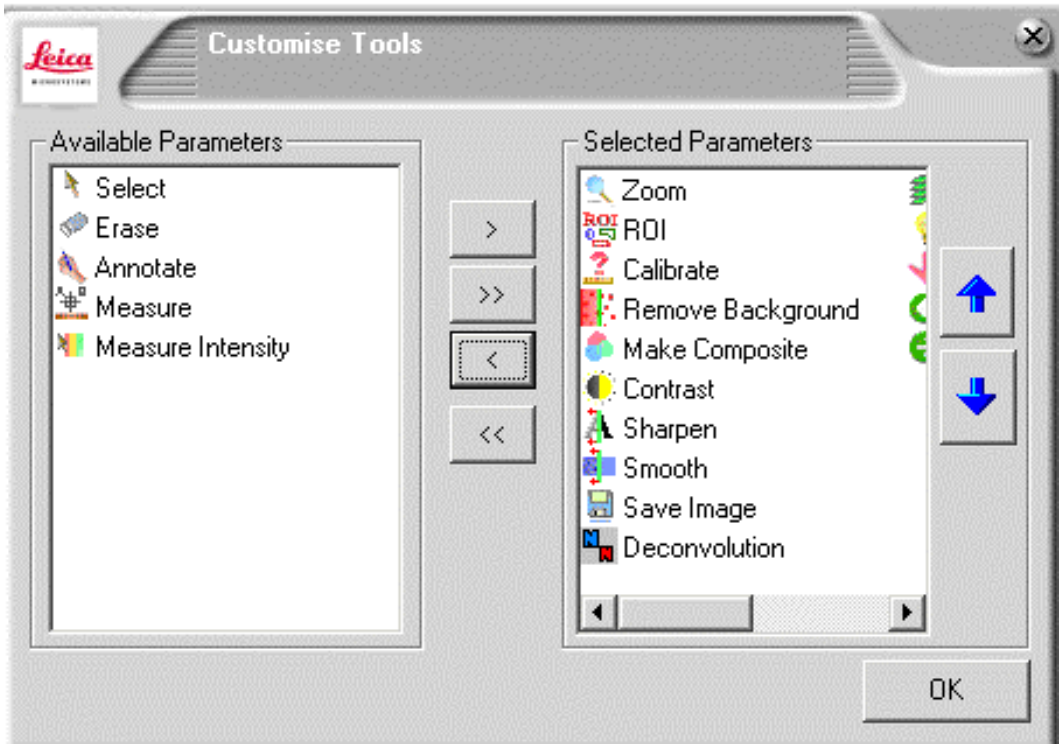
Customising Your Image Toolbar

You may add and remove toolbar buttons, and therefore functionality, from the image toolbar.

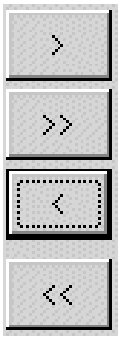
To customise your image toolbar:

1. Click **Settings** in the main menu and click **Customise Toolbar**. This displays the **Customise Tools** dialog.



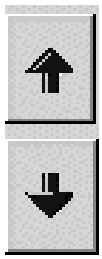


2. Move toolbar parameters between the two lists until the set of tools you require for your experiment is listed in **the Selected Parameters** list. Use the single and double arrows in the centre of the dialog to move the parameters.



Note: Single arrows move the highlight parameter in the direction of the arrow. Double arrows move the entire contents of the selected list in the direction of the arrow.

3. Move the parameters to the desired order in the **Selected Parameters** list by using the up and down arrows.
4. Click **OK**.



Using the Processed Image Set

When you create a new experiment in the Lab Book, an image set called 'Processed Images' is created automatically in the experiment. This is the default image set used when the **Saving Images** dialog appears. There are a number of places where this dialog is used after clicking **Apply** or **Apply to all** when using the deconvolution or image enhancement tools.

Once you choose where the processed images should go, the image set that is currently 'in session' changes in the drop-down list at the bottom left of the image viewer. This provides a mechanism for you to switch image sets from the **Image Viewer**. The new image sets are also available in the gallery and in the tree view in the Publish Results dialog.

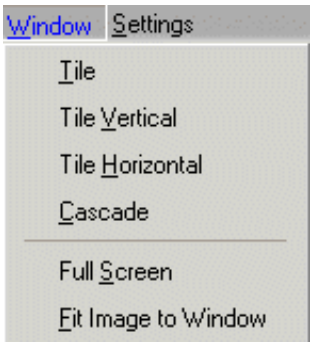
This dialog appears after clicking on **Apply** or **Apply to all** in the Image Viewer when you are using the deconvolution or image enhancement tools.

Windowing tools

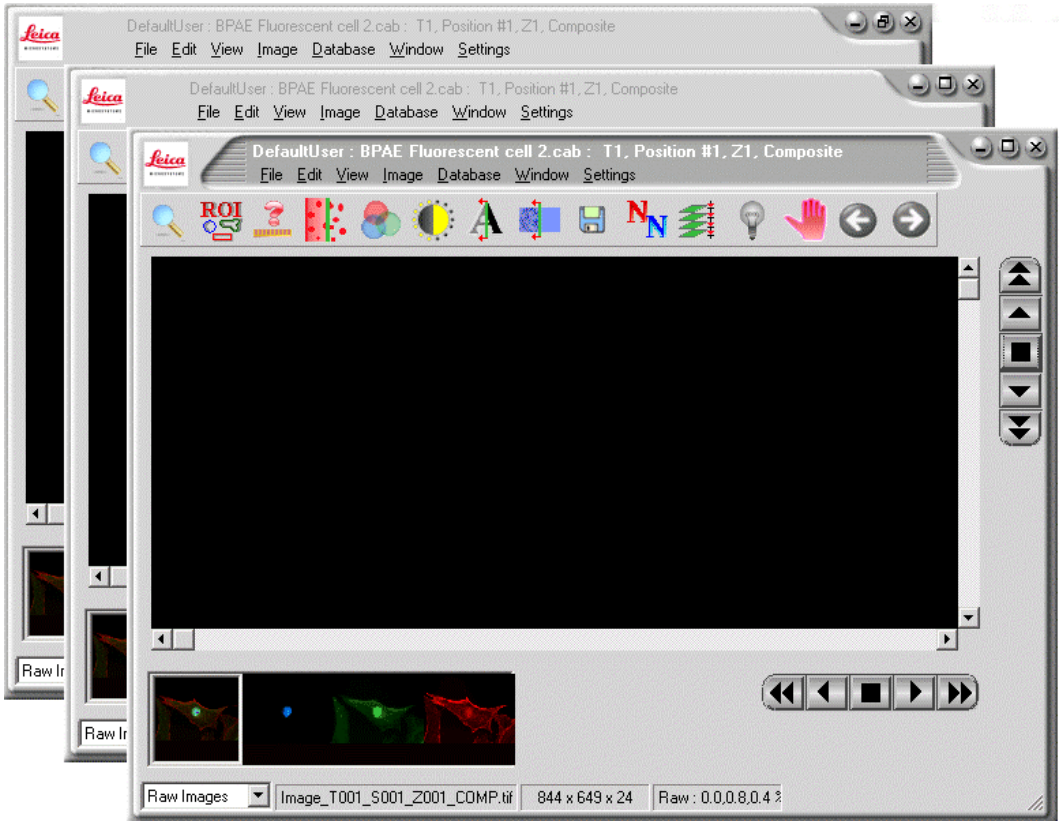
The **Window** menu allows you to cascade, arrange image windows, arrange buttons, fit images to the current window (size) or fit the window to the current image and close windows.

To cascade and tile windows:

1. Open the **Leica Image Viewer**.
2. Select the **Window** menu.
3. Click the appropriate function.



➡ **Cascade** arranges all open **Leica FW4000** windows in a cascading overlapping pattern. The title bars of the windows remain visible.


DefaultUser : BPAE Fluorescent cell 2.cab : T1, Position #1, Z1, Composite
File Edit View Image Database Window Settings
ROI
Raw Images
Image_T001_S001_Z001_COMP.tif
844 x 649 x 24

➡ **Tile Horizontal** arranges all open **Leica FW4000** windows in a non-overlapping side-by side pattern, which allows you to view all the windows at once.

➡ **Tile Vertical** arranges all open **Leica FW4000** windows in a non-overlapping horizontal pattern, one above the other, which allows you to view all the windows at once.

➡ Full screen displays only the active **Leica FW4000** window as a full-screen image.

➡ **Fit Image to Window** adjusts the image size to fit into the Image Viewer window.

Zoom

➡ To magnify an image by zooming, click the **Zoom** button in the **Image Toolbar**.

As you left-click in the image, the image size is increased so that you can see it in more detail. Conversely, as you right-click in the image, the image size is decreased so you can see more of the image in one screen. If the image is too big for the image window, use the scroll bars to move around in the image.

➡ Alternatively, use the **Pan** facility to move the image around in the image window. Click the **Pan** button in the **Image Toolbar**. You can then click part of the image and drag it within the image window.
To switch off **Pan** mode, click the **Pan** button again.

Erasing

To erase a defined area:

1. Click the **Erase** button in the **Image Toolbar**.

2. Define an area to be erased by holding down the left mouse button and drawing around the required area.
3. Release the mouse button to delete the image area defined.

Send Images to Leica QWin

Leica QWin exports the current image to **Leica's QWin** image processing application for further detailed measurement and analysis. **Leica QWin** is available for purchase separately.

Note: You should have **Leica QWin** installed and running **before** you use this option.

No Neighbours and Nearest Neighbours Deconvolution

Note: The **3D deconvolution by nearest neighbour** is a **Leica FW4000** product option that is only available if you have purchased the **FW4000Z** or **FW4000TZ** modules.

There are two types of deconvolution:

- No Neighbours and Nearest Neighbours, which are accessed from the Image viewer and discussed in this chapter.
- Deconvolution offered in **Leica Deblur and 3D Visualise**, which pops up when you click on the process arrow.

Refer to: *Chapter 10, Advanced Deconvolution* for more detailed information on using deconvolution techniques.

Refer to: *Chapter 2, Point Spread Functions Management* for more information on the PSF dialog.

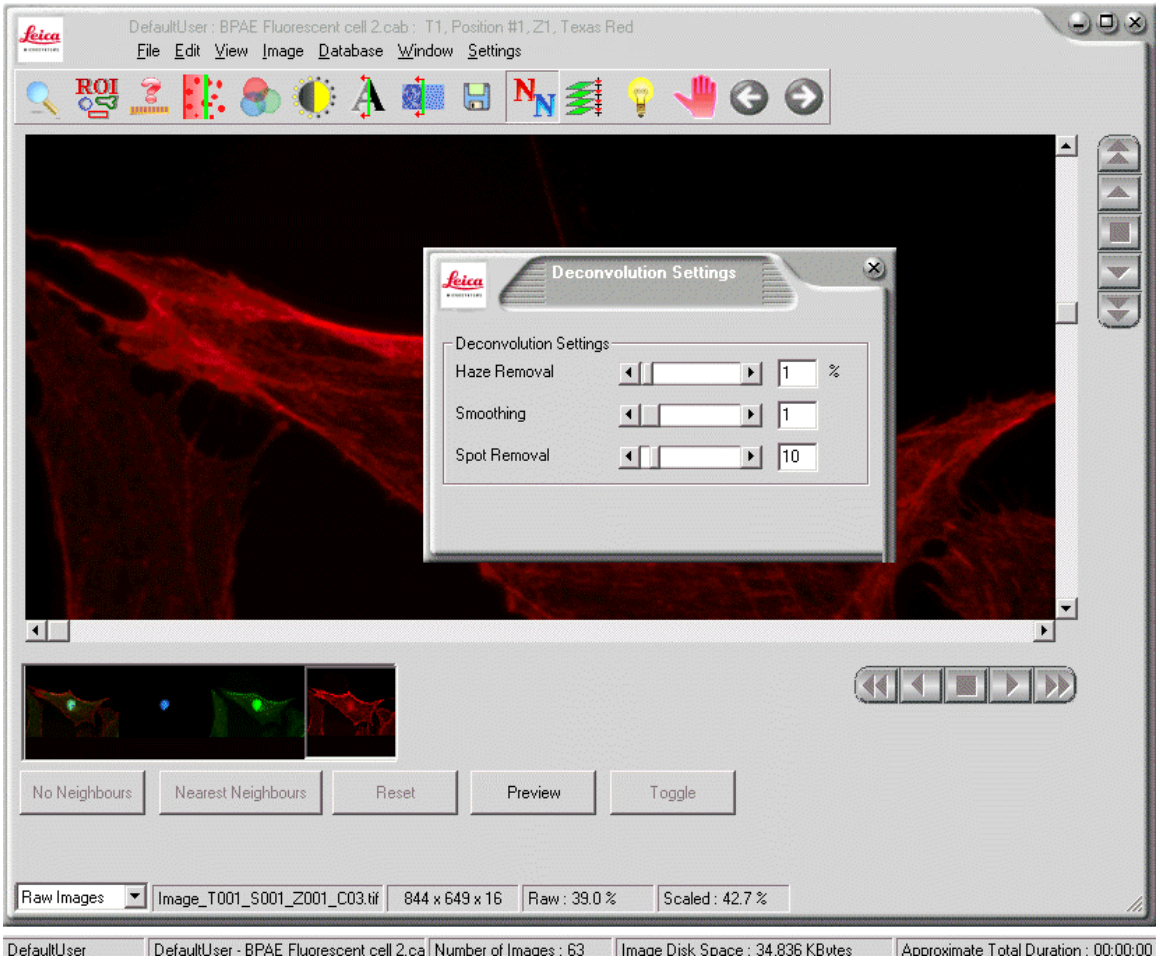
The Deconvolution button allows you to apply both No Neighbours and Nearest Neighbours deconvolution. The difference in operation between the two types of deconvolution is:

- No neighbours deconvolution applies to one image
- Nearest neighbours deconvolution applies the selected parameters to the selected stack

To perform deconvolution on an image:

1. Open the **Image Viewer** dialog.
2. Select the **Image** menu; click **Deconvolution** to display the **Deconvolution Settings** dialog.

➡ Alternatively, click **Deconvolution** in the **Image toolbar** to display the **Deconvolution Settings** dialog.



3. Specify the amounts of **Haze Removal** and **Smoothing** by using the sliders or by typing directly into the boxes.
4. Click **Preview** to display the results of the operation in the main image window without changing the image permanently.
5. Click and hold down **Toggle** to view the original image. Use this button to compare the image before and after the enhancement.
6. Click **No Neighbours** or **Nearest Neighbours** to display the **Saving Images dialog**.

7. Specify an existing image set, or create a new image set, to which you want to add your images.
8. Click **OK** to close the dialog and apply the changes, or click **Cancel** to close the dialog without saving any changes.

Note: When you create a new experiment in the Lab Book, an image set called 'Processed Images' is created automatically in the experiment. This is the default image set used when the **Saving Images** dialog appears.

Refer to: *Chapter 7, Using a Processed Image Set* for further information.

Defining a Region of Interest

All image enhancement operations, apart from background removal, can be applied either to the whole image or to a Region of Interest (ROI). You can define the ROI to encompass any areas or features in the component image in which you are particularly interested.

There are two methods of defining an ROI:

- By using the manual image processing tools through the **ROI** option in the **Settings** main menu or the Image Toolbar. The types of region you are recommended to set manually include straight, rough outline or a small number of regions. You may only enhance the images in the ROI defined using this method.
- Features defined using the **Define Ftr** button on the bottom of the Measurement dialog. You are recommended to use a feature to define complex regions, or regions comprising multiple objects. You may measure and enhance ROI when using the **Define Ftr** button.

The **Define Ftr** button allows you to assign colours, line thickness and object names to regions.

The purpose of accessing the **ROI** dialog from within the measurements area is to use the areas defined for feature measurement, as opposed to image processing.

Note: ROI can only be defined on component images. However, if you choose **Use ROI** from the **Settings** main menu, the first of the component images is selected automatically. You can select the required component image by clicking it in the **Image Gallery**.

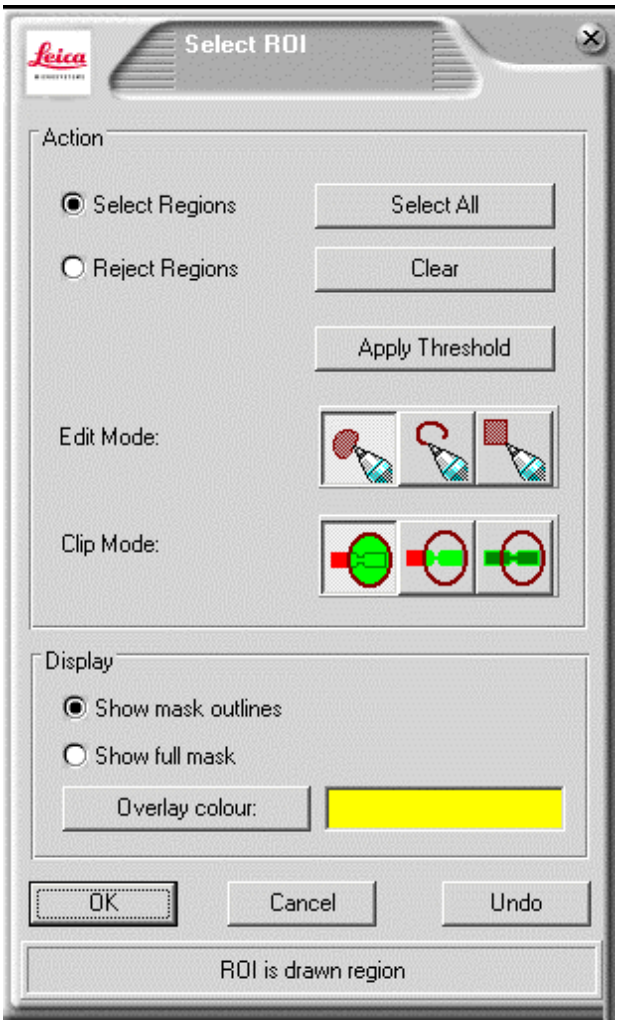
Note: You must remove the background from an image before you can define a region of interest. If you have not done this already, you will be prompted when you click **OK**.

Refer to: *Chapter 9, Removing the Background* for information on removing the background from an image.

The **Select ROI** dialog allows you to select a sub-region of the image. This means that you can limit processing to specific parts of the image by using an **ROI processing mask**. The ROI mask tells **Leica FW4000** which pixels are to be affected during processing of the image. You can define any number of shapes in the ROI mask using simple mouse editing. The resulting mask image can be thought of as a transparency, which can be placed over the image. You then specify which parts of the image you are interested in by drawing on the transparency.

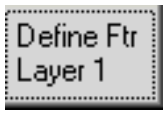
To define a region of interest:

1. Select the required component image.
2. From the **Image** main menu, choose **Setup ROI**. The **Select ROI** dialog appears.



➡ Alternatively, click the **ROI** icon in the image toolbar.

➡ Alternatively, from the Measurement dialog, click the **Define Ftr Layer 1** button.

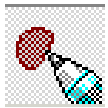


3. Select the image to which the ROI will be referenced from the drop-down list. This image will be displayed during the ROI definition procedure. For the editing modes, which limit the ROI to the feature boundary, this also defines the image that is used as the feature mask.
4. In the **Action** area of the dialog, specify whether you are adding or removing regions from the ROI:

Select Regions	Adds the regions you defined to the ROI mask.
Reject Regions	Removes defined regions from the ROI mask.
Select All	Add the entire image to the ROI mask.
Clear	Removes all regions from the ROI mask.
Apply Threshold	Identifies the areas of an image which are objects of interest or background, but does not actually remove the background

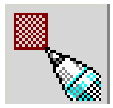
Refer to: *Chapter 9, Removing the Background* gives details on removing the background tools.

5. Select the appropriate tool for defining the required region of interest:
   - ♦ Draw a freehand filled region.



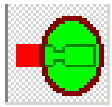
   - ♦ Draw a line.

- Draw a filled square.

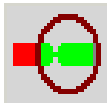


6. Select the **Clip Mode**:

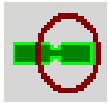
- Specify the full area, as drawn.



- Clip the drawing to the area of overlap with the feature mask; that is, include only the feature(s) specified by the ROI mask, not the background.



- Clip the drawing to the entire feature connected to the ROI; that is, if part of a feature is inside the ROI, the whole of that feature will be included in the ROI mask.



7. You can now draw with the selected tool on the image to define a region. You can define several regions if necessary.
8. Click **OK** to close the **Select ROI** dialog.

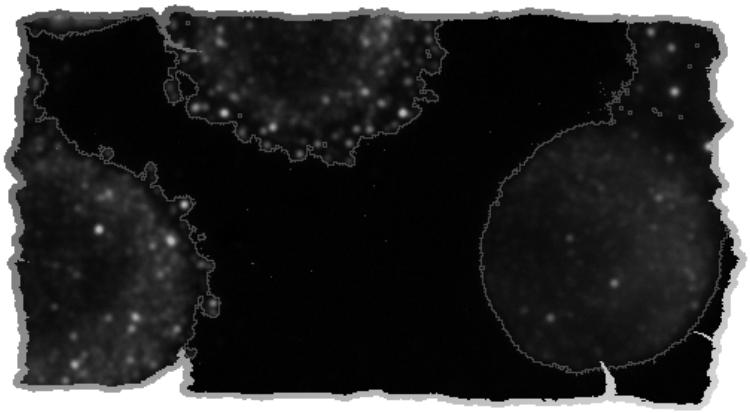
Note: The information area at the bottom of the dialog will display information about the selected modes.

9. Use the **Display** area of the dialog to specify how the ROI mask should be displayed on the image:

Show mask outlines	Displays only the outlines of included features.
Show full mask	Displays the entire features.

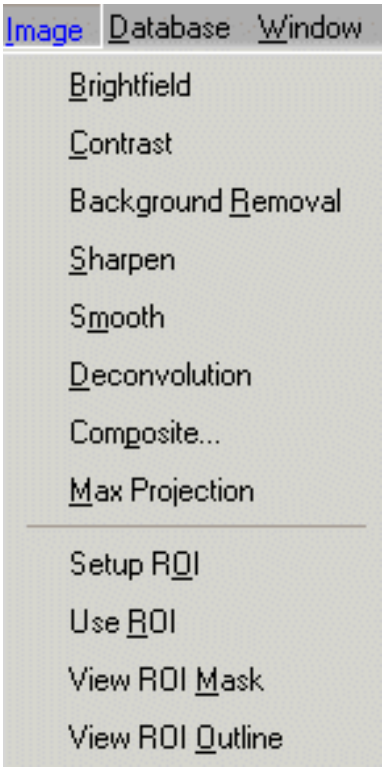
10. Click **Overlay Colour** to change the colour in which the ROI mask appears. This will display the **Colour** dialog. Click a colour and click **OK** to close the **Colour** dialog to apply the new colour.
11. Click **OK** to close the dialog, and apply the ROI mask. Click **Cancel** to close the dialog without saving the changes. Click **Undo** to cancel the most recent operation.

Below you can see an example of features selected by an ROI mask. The lines around the features indicated the defined regions.



Applying an Overlay

➡ Click on the **Image** in the main menu and select the type of overlay display you require for your experiment from one of the options:



Setup ROI Shows the objects detected once you have defined the background threshold level using the **Remove Background** dialog.

View ROI Mask	Shows the current region of interest (ROI) mask. This mask may be applied to limit image-processing operations to only those areas of the image under the mask.
View ROI Outline	Displays only the edges of the current ROI.

➡ Set **Full Object Detection** by selecting **Image** from the main menu, and clicking **Background Removal**.
Alternatively, click **Apply Threshold** in the **Select ROI** dialog.

Refer to: The section, *Removing the Background* in *Chapter 9* for information on removing the background from an image.

Note: You are able to **Load** and **Save** regions by using the **File** menu in the **Measurements** dialog.

Chapter Eight: Performing measurements

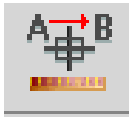
Chapter Overview

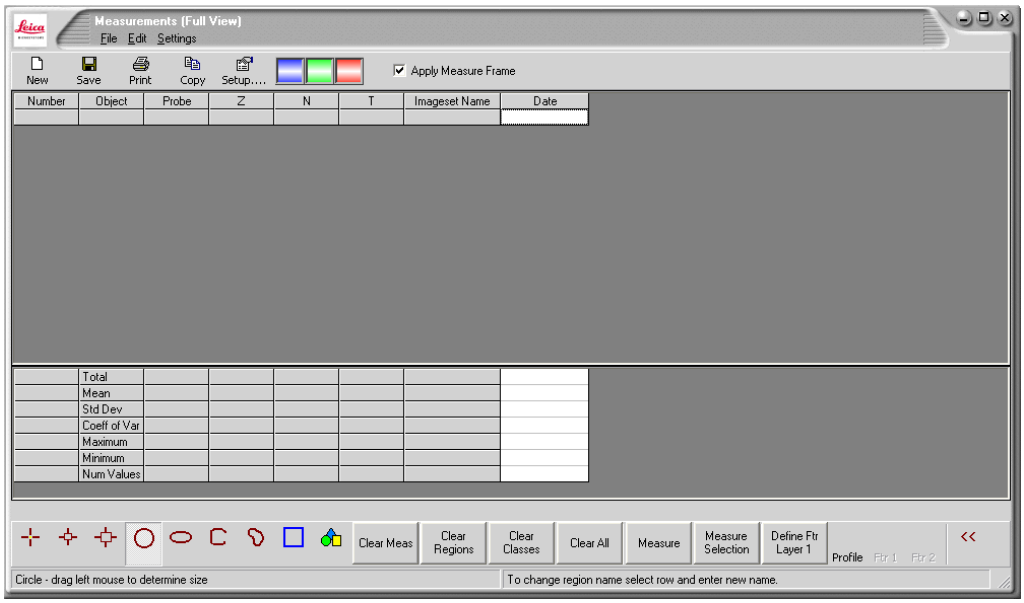
This chapter contains the following topics:

- Using the Measurements Dialog
- Specifying Measurement Parameters
- Changing the Colour and Thickness of Lines
- Changing the Number Format
- Making Measurements Using the Feature Layer
  - Changing the Name of a Region
  - Changing the Colour of a Region
  - Clearing Regions of Interest
  - Saving a Region
  - Deleting a Region
- Synchronising the Set of Results with Excel
- Making a Measurement Manually
- Measuring Probe Intensity
- Calibrating an Image
- Annotating your Image
  - Adding an Annotation
  - Changing the Annotation Toolbox Settings
  - Displaying Annotations
  - Clearing Annotations
  - Saving Annotation
  - Measuring an Angle
- Printing a Set of Results

Using the Measurements Dialog

➡ Click the **Measurement** button in the **Image Toolbar** to open the **Measurements** dialog.





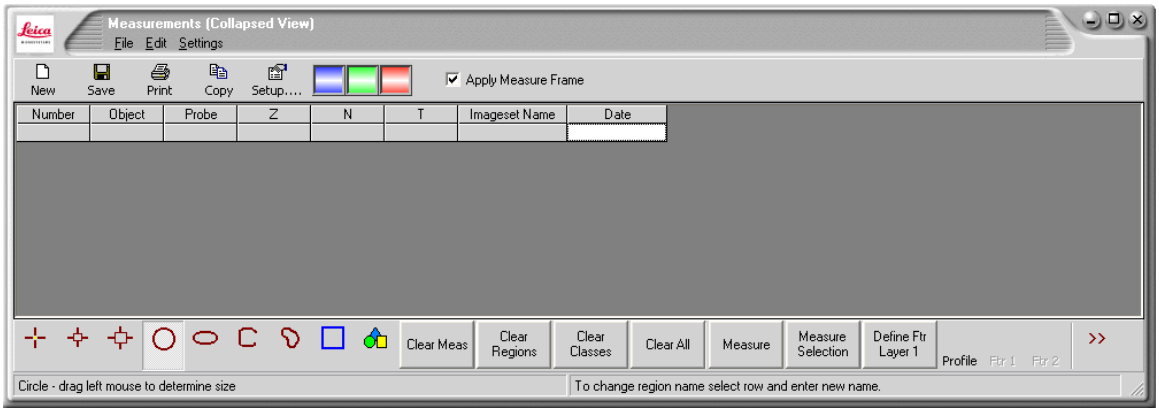
The measurements dialog allows you to perform measurements within the image and view the results on the screen. You can also save and load results, export them to Microsoft Excel and print them using an Excel template. Excel must be installed for these options to be available.

The measurement selection allows you to apply the measurement criteria to the images selected in the gallery.

The identity of the image is shown in the measurement table, in the Z, N or T column. This activity may produce many measurements that may be filtered by probe.

If required, you can collapse the **Measurements** dialog to make it easier to draw on the image.

➡ Click the **Shrink** button to shrink the **Measurements** dialog. The dialog will shrink to contain a reduced set of data.



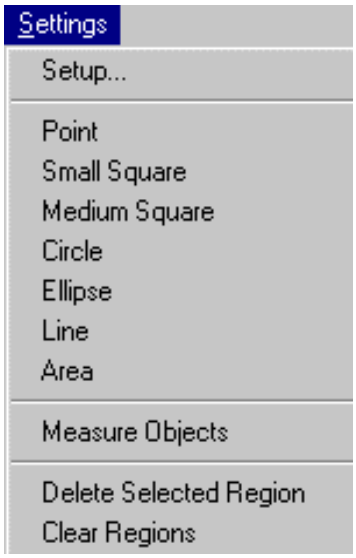
➡ Click the **Enlarge** button to enlarge the **Measurements** dialog. The dialog will enlarge to contain the full set of data.

Specifying Measurement Parameters

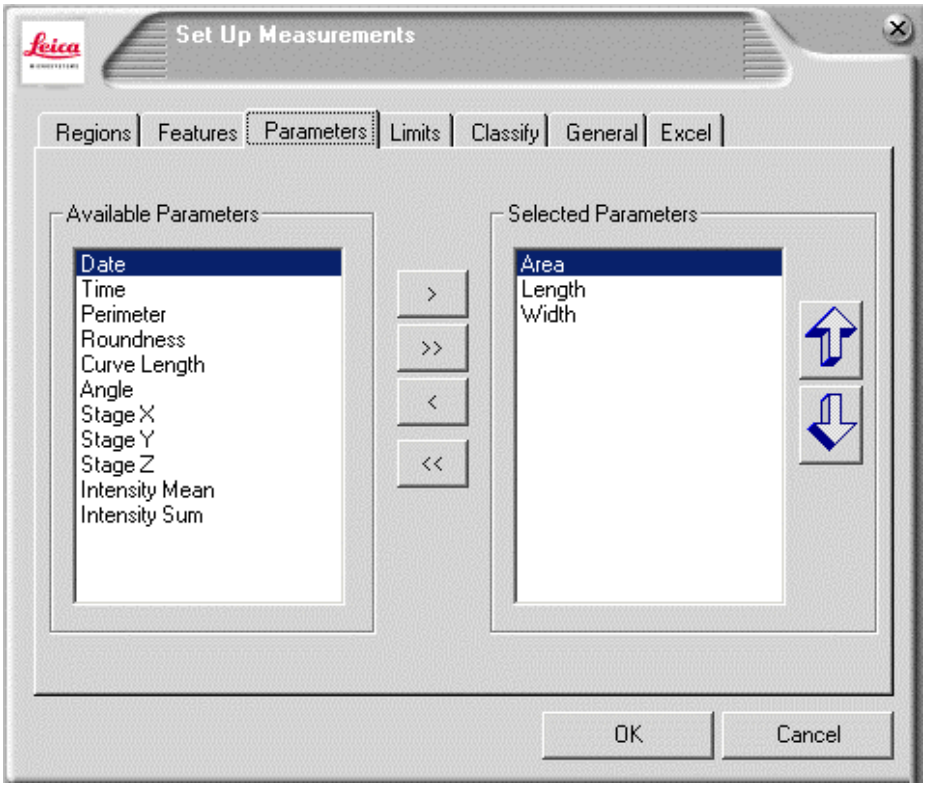
To specify the parameters you want to measure:

1. Click the **Setup** button in the **Measurements** dialog to display the **Set Up Measurements** dialog.

➡ Alternatively, click **Settings** in the **Measurement** dialog, and choose **Setup**.



2. Select the **Parameters** tab.



The **Parameters** tab allows you to specify the parameters that you want to measure. The **Available Parameters** list displays a list of parameters that are currently not in use. The **Selected Parameters** list displays the parameters that are currently in use.

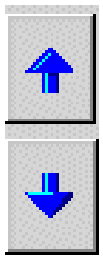
- To select an available measurement parameter, click the required parameter. Click the **Select** button to move it to the list of selected parameters.

- To deselect an available measurement parameter, click the required parameter. Click the **Deselect** button to move it to the list of available parameters.

- To select all available measurement parameters, click the **Select all** button to move all available parameters to the list of selected parameters.

- To deselect all available measurement parameters, click the **Deselect all** button to move all selected parameters to the list of available parameters.

➡ To change the order of parameters in the selected list, select a parameter you wish to move, and click either of the up or down arrows, as appropriate.



➡ Click **OK** to close the dialog saving the changes, or **Cancel** to close the dialog without saving any changes.

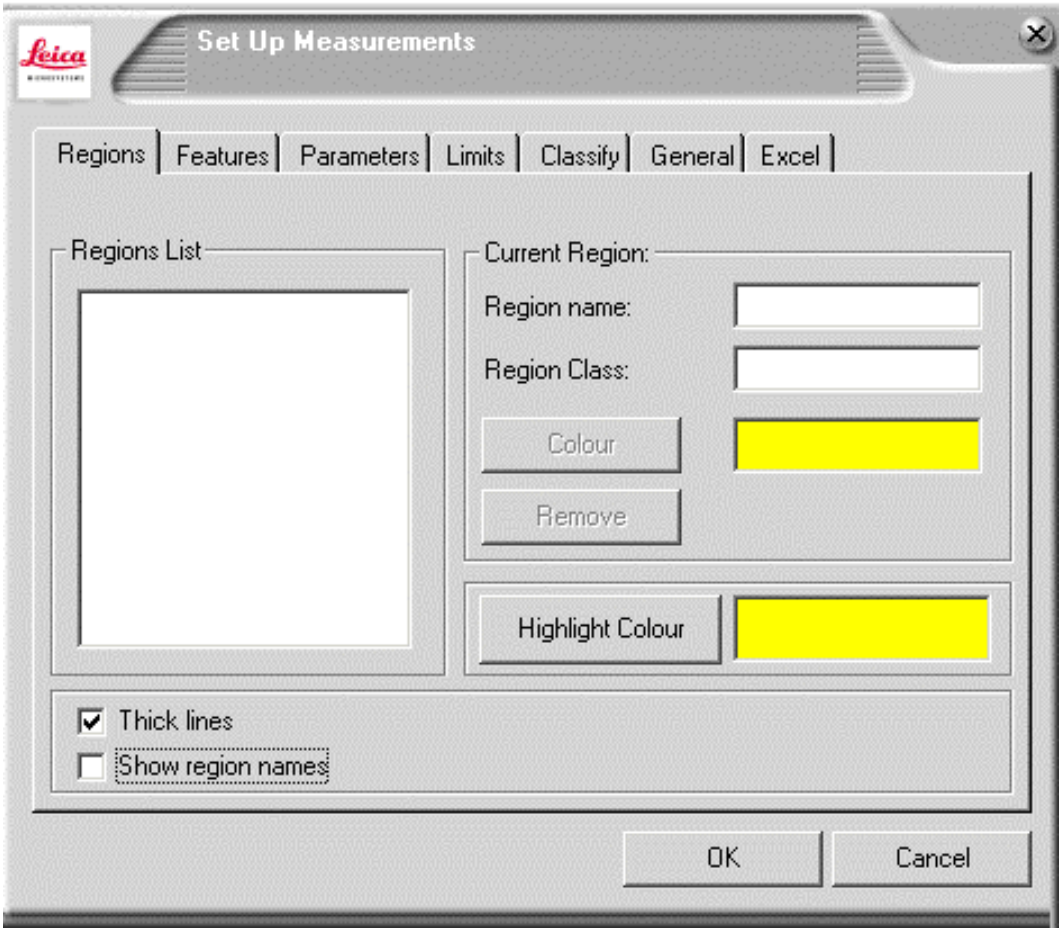
Changing the Colour and Thickness of Lines

To change the colour and thickness of lines:

1. Click on the **Setup** button in the **Measurements** dialog to display the **Set Up Measurements** dialog.

Alternatively, click **Settings** in the **Measurement** dialog, and choose **Setup**.

2. Click on the **Regions** tab.



3. Click the **Highlight Colour** button to display the **Colour** dialog.
4. Select the required colour.
5. Click **OK** to close the **Colour** dialog.
6. Check **Thick Lines** if you want the lines to appear thicker.

7. Click **OK** to close the dialog and save the changes, or **Cancel** to close the dialog without saving any changes.

➡ To use thick line when drawing your measurement line, check the **Thick lines** box.

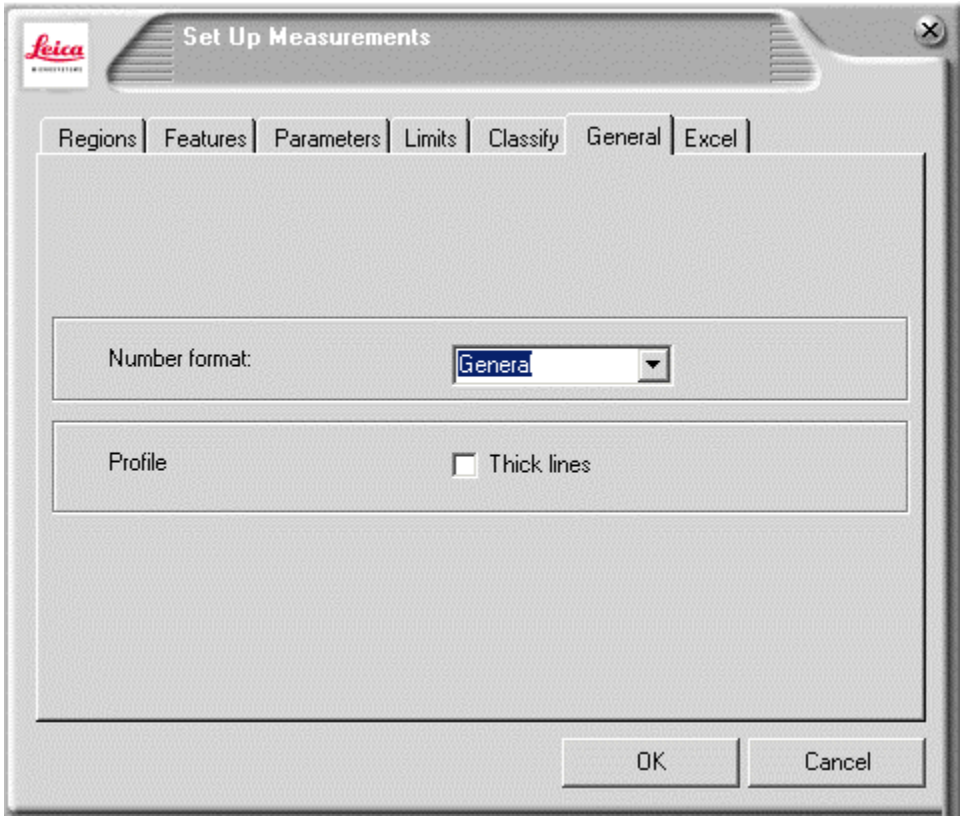
Changing the Number Format

To change the number format:

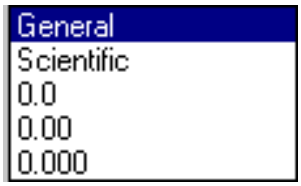
1. Click on the **Setup** button in the **Measurements** dialog to display the **Set Up Measurements** dialog.

➡ Alternatively, click **Settings** in the **Measurement** dialog, and choose **Setup**.

2. Click on the **General** tab.



3. Click the **Number format** menu button to display the available formats.
4. Choose a number format from the drop-down list.



5. Click **OK** to close the dialog and save the changes, or **Cancel** to close the dialog without saving any changes.

Making Measurements Using the Feature Layer

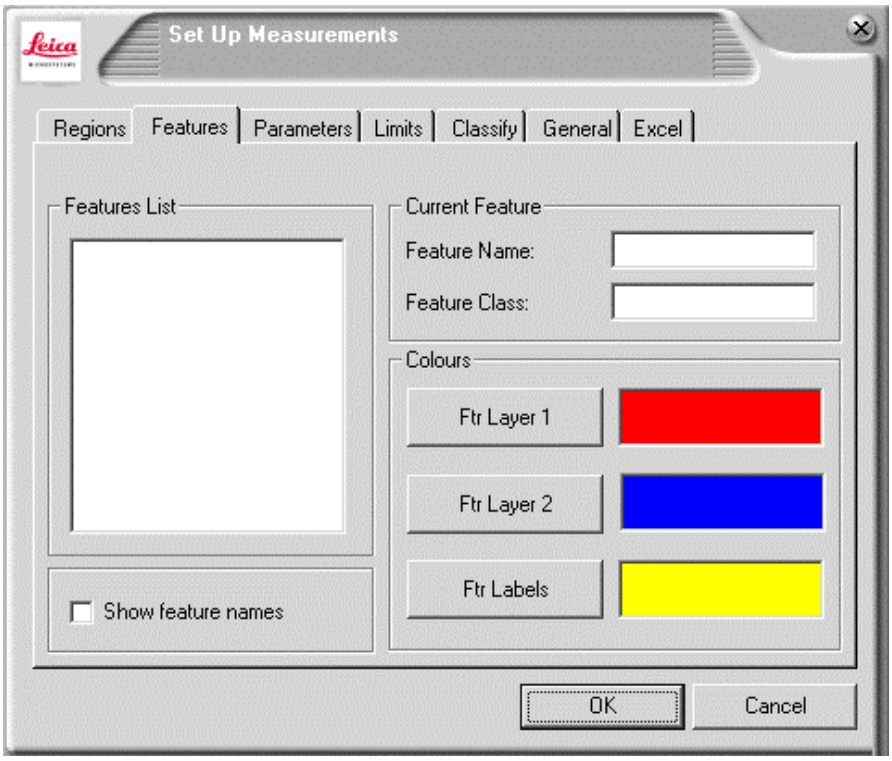
Using the Feature Layer to define a region of interest allows you to define complex and large regions for a variety of probes.

To make a measurement using the Feature Layer:

1. Click the **Setup** button in the **Measurements** dialog to display the **Set Up Measurements** dialog.

➡ Alternatively, click **Settings** in the **Measurement** dialog, and choose **Setup**.

2. Click on the **Features** tab.

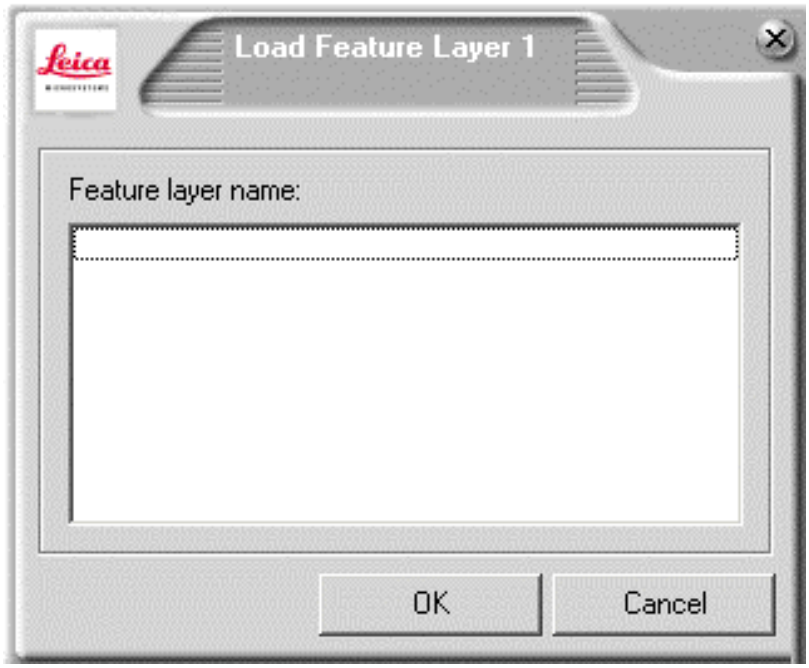


3. Set the fields as appropriate for the measurement you wish to make.
4. Click **OK** to close the dialog and save the changes, or **Cancel** to close the dialog without saving any changes.

➡ Check **Show feature names** to display the measurements for all the features defined as ROIs in the image.

To select and load a Feature Layer:

1. Click **File** in the Measurement dialog.
2. **Select Load Feature Layer n**, to display the Load Feature Layer dialog.



3. Select the feature name required.
4. Click **OK** to close the dialog and load the selected ROIs, or **Cancel** to close the dialog without loading any ROIs.

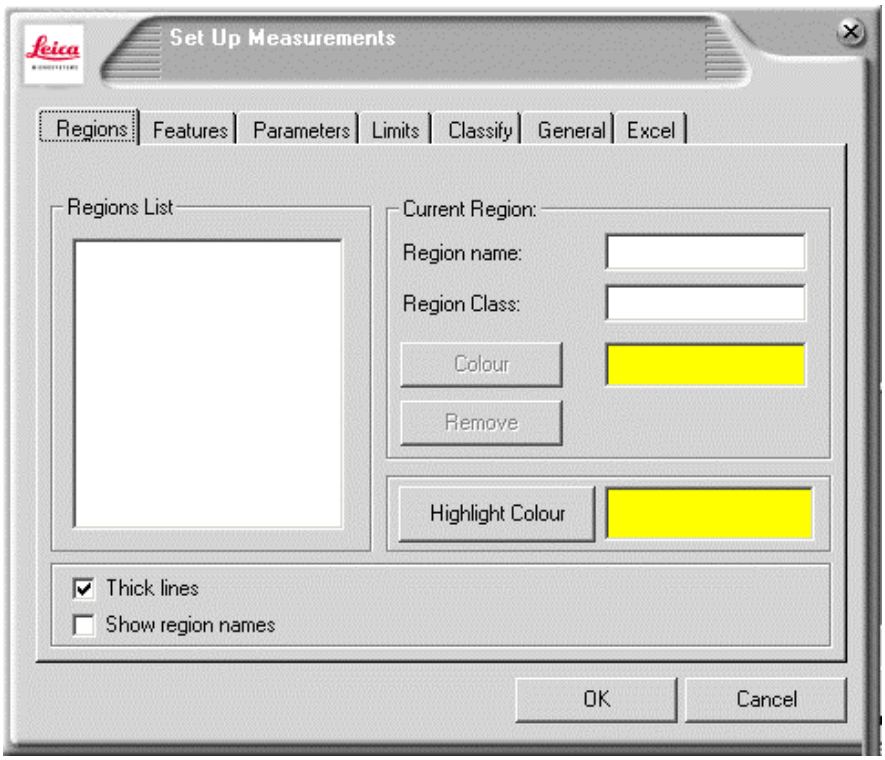
Changing the Name of a Region

To change the name of a Region:

1. Click the **Setup** button in the **Measurements** dialog to display the **Set Up Measurements** dialog.

➡ Alternatively, highlight the name of the region and type the new name.

2. Click on the **Regions** tab.



3. Click on the **Region name** in the dialog.
4. The **Regions List** contains a list of all the regions, in the order that they were created.
5. Type in the new name.
6. Select the required region from the list.
7. Type a name into the **Region name** box.
8. Click **OK** to apply the changes and close the dialog, or click **Cancel** to close the dialog without applying the changes.

➡ Alternatively, to change the name of a region, highlight the name of the region, and overtype the new name.

Changing the Colour of a Region

To change the colour of a region:

1. Click the **Setup** button in the **Measurements** dialog to display the **Set Up Measurements** dialog.

➡ Alternatively, right click the colour, and select a new colour from the displayed colour palette

2. Select on the **Regions** tab.
3. Select the **Region name**.

Note: The **Regions List** contains a list of all the regions, in the order that they were created.

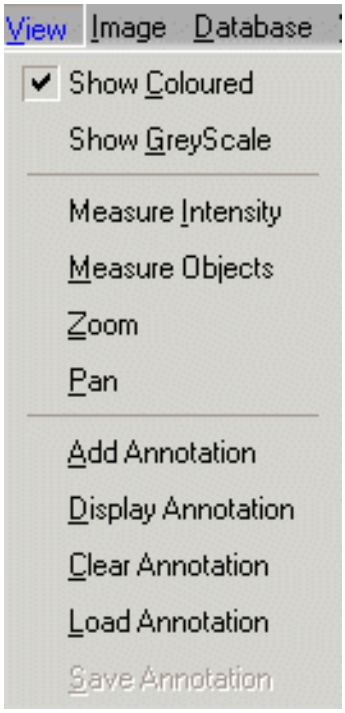
4. Right-click on the region name to display the **Colour** dialog.
5. Select the required colour.
6. Click **OK** to close the **Colour** dialog.
7. Click **OK** to apply the changes and close the **Set Up Measurements** dialog, or **Cancel** to close the dialog without applying the changes.

➡ Alternatively, to change the colour of a region, right click in the region, and select the new colour from the colour palette.

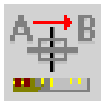
Clearing Regions of Interest

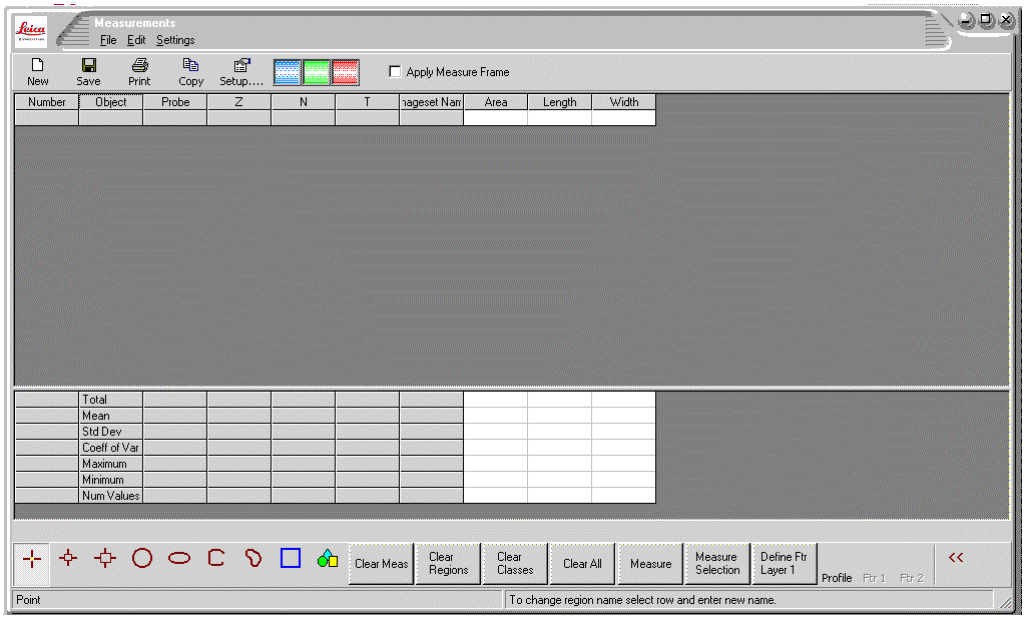
To clear a region:

1. Click **View** in the main menu and choose **Measure Objects** to display the **Measurement** dialog.

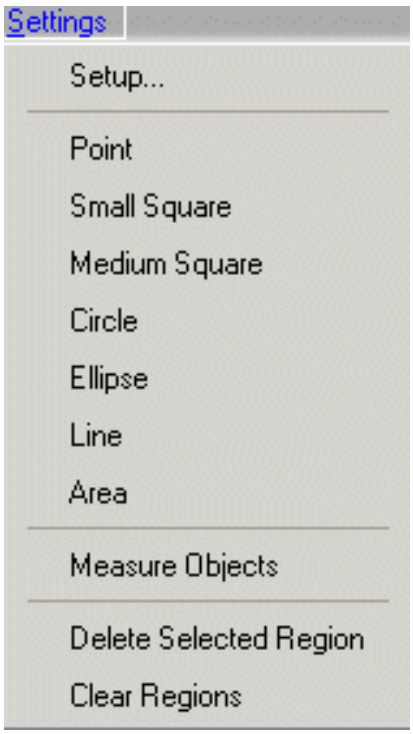


➡ Alternatively, click the **Measurements** icon.





2. Click **Settings** in the main menu for the dialog.
3. Click **Clear Regions**.

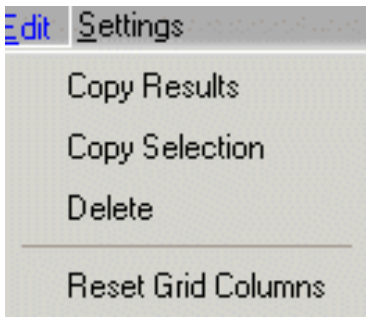


Saving a Region

➡ To save a set of measurements, click **Save** in the main toolbar in the **Measurements** dialog.

Deleting a Region

➡ To delete a region, select the required region in the **Measurements** dialog, open the **Edit** menu, and select **Delete**.



Synchronising the Set of Results with Excel

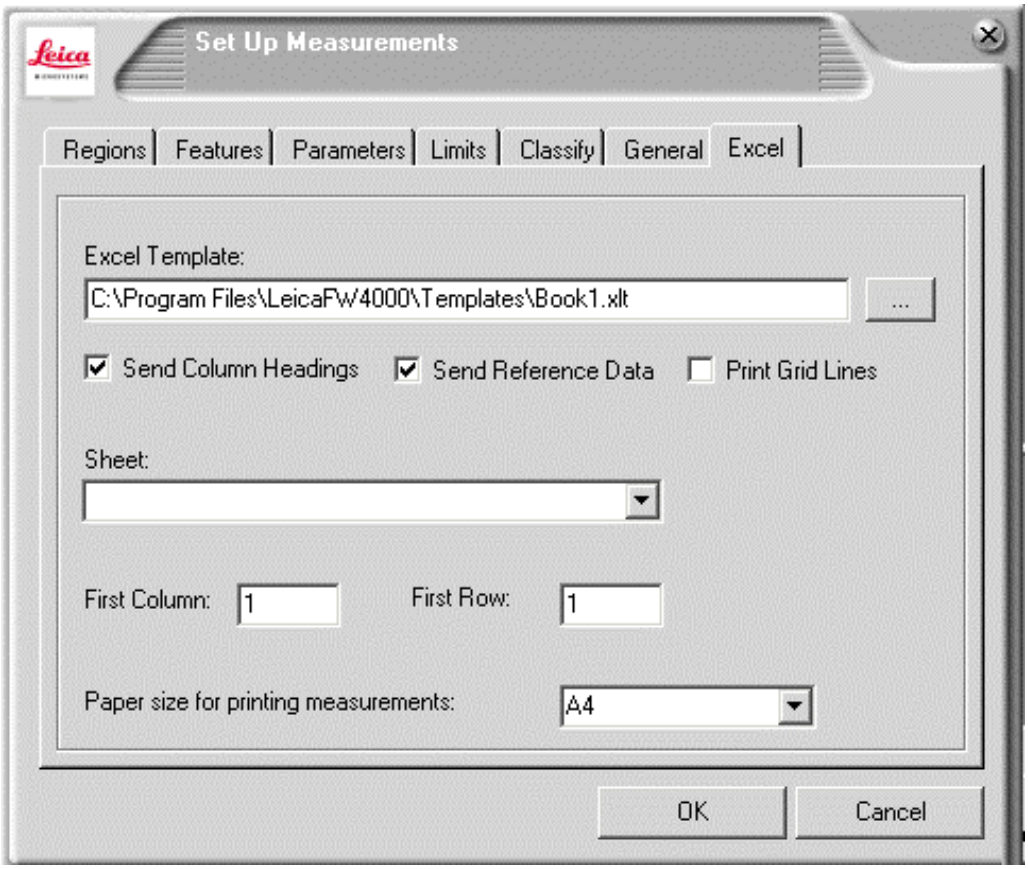
The structure of the measurements being taken is compatible with your Excel spreadsheet.

To change the Excel template:

1. Click the **Setup** button in the **Measurements** Dialog to open the **Set Up Measurements** dialog.

➡ Alternatively, click **Settings** in the **Measurement** dialog, and choose **Setup**.

2. Select the **Excel** tab.



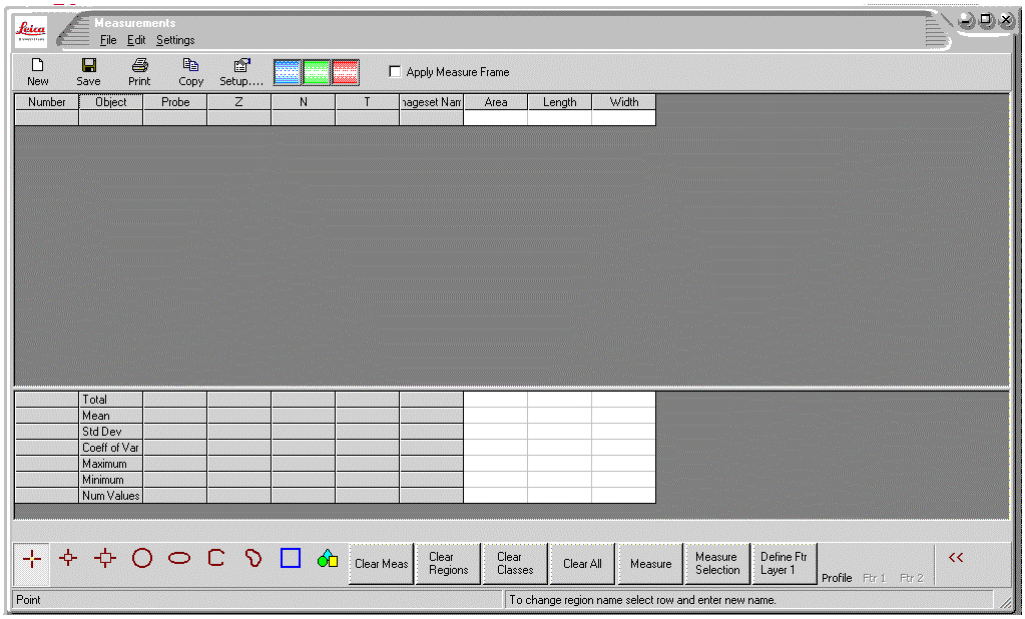
3. Type the name of the required **Excel Template** into the Excel Template box.
4. Alternatively, click the **...** button to browse to locate the required template.

5. Check **Send Column Headings** if required.
6. For templates that use more than one **Sheet**, select the sheet from the drop-down list.
7. Specify the **First Column** and **First Row** numbers if required.
8. Click **OK** to apply the changes and close the dialog, or **Cancel** to close the dialog without applying the changes.

Note: You are able to **Save** the Excel files by using the **File** menu in the **Measurements** dialog.

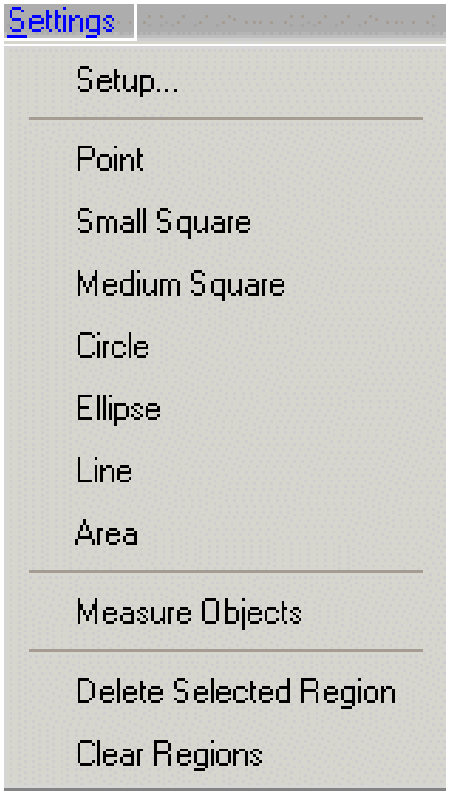
Making a Measurement Manually

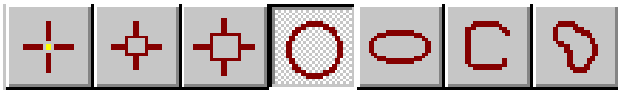
➡ Use the toolbar along the bottom of the **Measurements** dialog to select the kind of shape you want to draw on the image.



➡ Alternatively, click **Settings** in the **Measurement** dialog, and choose **Setup**.

Click on the appropriate menu item to perform the action you desire. The actions from these menu selections are identical to the actions launched by the buttons described below.





➡ Select the **Point** button, and then click the required point in the image.

➡ Select the **Small square** button, and then click the required point in the image.

➡ Select the **Medium square** button, and then click the required point in the image.

To draw a circle:

1. Select the **Circle** button.

2. Click in the image where you want the top-left-most point of the circle to be. **Do not** release the mouse button.
3. Hold the left mouse button down and drag the circle to the required size.
4. Release the mouse button to finish.

To draw an ellipse:

1. Select the **Ellipse** button.

2. Click in the image where you want the top-left-most point of the ellipse to be. **Do not** release the mouse button.
3. Hold the left-mouse button down and drag the ellipse to the required size.
4. Release the mouse button to finish.

To draw a line:

1. Select the Line button.
2. Click in the image where you want the line to start, by either:
   - Holding the left-mouse button down and draw a freehand line.
   - Releasing the mouse button and click again to draw a straight line. You can do this many times to create a number of nodes joined by straight lines.
   - You can also combine these two methods if required.
3. Release the mouse button to finish.

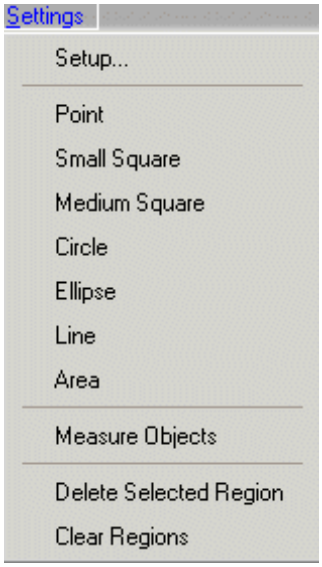
To draw an area:

1. Select the **Area** button.
2. Click in the image where you want the area to start, by either:
   - Holding down the left-mouse button and draw a freehand area.
   - Releasing the mouse button and click again to draw a straight area. You can do this many times to create a number of nodes joined by straight lines.
   - You can also combine these two methods if required.
3. Click the right-mouse button to finish drawing the area. The end of the line will automatically join with the start of the line.

As you finish drawing the shape, the measurements will appear in the **Measurements** dialog.

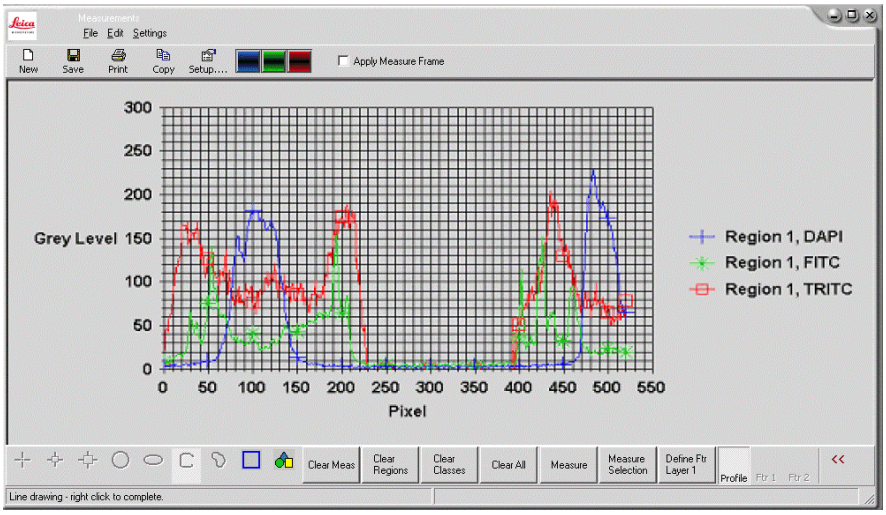
➡ To measure an object click the **Measure** button in the **Measurements** dialog.

➡ Alternatively, click **Settings** in the **Measurement** dialog, and choose **Measure Objects**.



➡ To measure the grey scale levels, click the **Profile** button in the **Measurements** dialog.

Note: The grey scale measurement tool is best used in conjunction with the line drawing tool.



Note: The **Profile** button toggles the grey scale level measuring tool on and off.

Note: You are able to **Load** and **Save** the profile by using the **File** menu in the **Measurements** dialog.

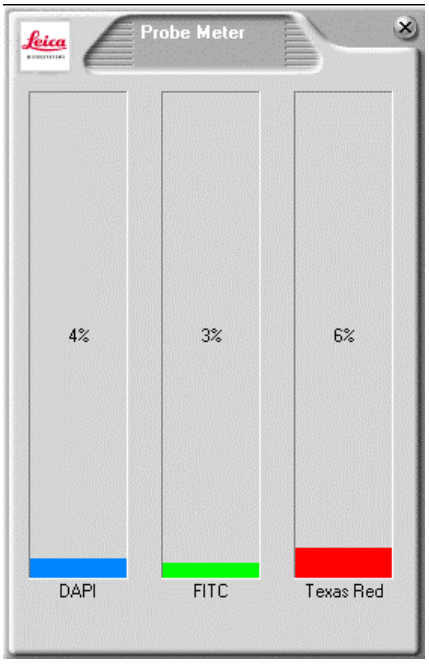
Measuring Probe Intensity

You can view the intensity levels of all the probes in the image stack at any point in the image.

To view the intensity level of a probe:

1. Select the required image in the Image Gallery.
2. Select **View** in the **Image Viewer** main menu and click **Measure intensity** to display the **Probe Meter** dialog.

Alternatively, click the **Measure Intensity** button in the **Image Toolbar** to display the **Probe Meter** dialog.



3. Click any point in the selected image to view the intensity for that point, or hold down the left mouse button and move the mouse around in the image to view the intensity levels at any point in the image instantaneously.
   The probes shown in this diagram will depend on the settings for the current image; that is, the filters that have been set up for this image.

Note: The current **X/Y position** is displayed in the **status bar** at the bottom of the **Image Viewer** dialog.

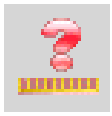
Calibrating an Image

If you wish to calibrate an image, you may use a stage micrometer and a live image, and draw a line corresponding to a known distance on the micrometer. When the mouse button is released, the **Calibration** dialog is displayed.

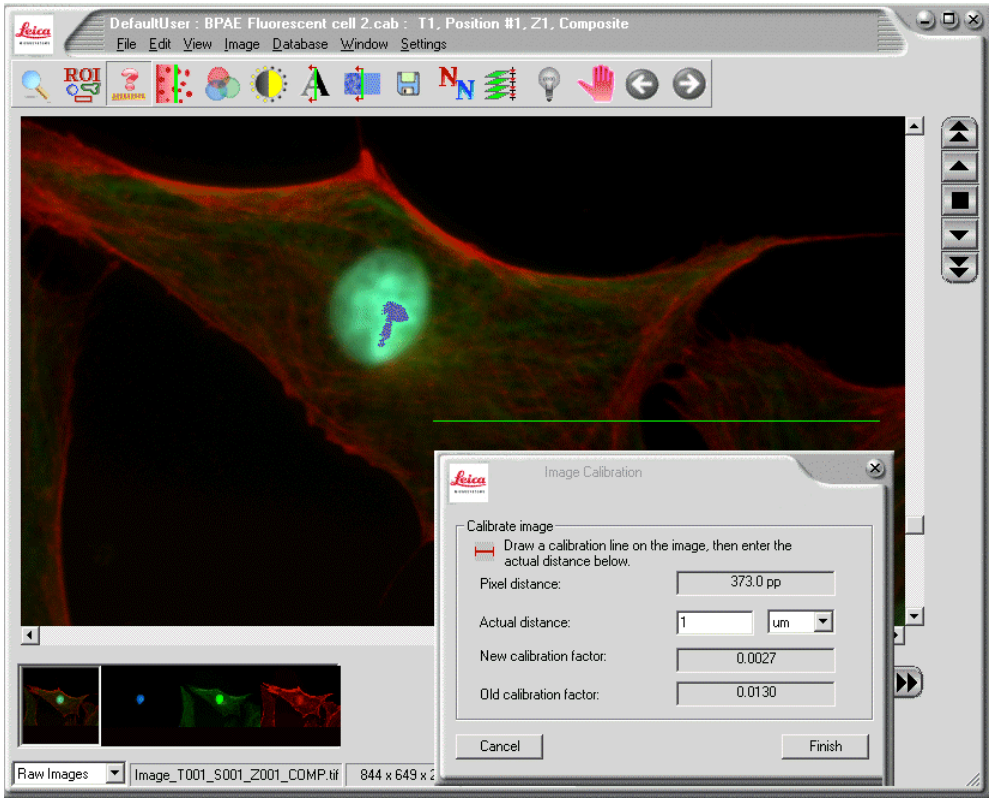
Note: When the system hardware is set-up correctly, FW4000 calibrates itself automatically on start-up.

To define image calibration:

1. Click the **Calibrate** button in the **Image Toolbar**.



2. Using a stage micrometer and a live image, draw a line (by clicking and dragging with the left mouse button) corresponding to a known distance on the micrometer. The mouse cursor is shown on the right side.
3. When the mouse button is released, the **Image Calibration** dialog is displayed and a calibration scale appears on the image.



4. Use the **Pixel distance** text box to type in the actual length of the calibration line you have just drawn.
5. Also, select the correct measurement **Unit**. You can choose from **Microns** (typical), **Millimetres**, **Centimetres**, **Nanometres** and I**nches**.
6. Click **OK** to apply the new settings or **Cancel** to abandon the changes.
7. You are then prompted to update the system calibration (as shown on the right side). Click **Yes** to update the calibration or **No** to retain the previous calibration settings.

Note: The **Calibration** button in the **Image toolbar** changes the calibration for the current image only.

Note: Only people with supervisor level access may calibrate the system. Users may calibrate the current image only.

Annotating your Image

The **Annotation Toolbox** provides facilities for you to annotate an image. You access the Annotation toolbox by clicking the **Annotation** button on the image toolbar.

The buttons in the annotation toolbox are:

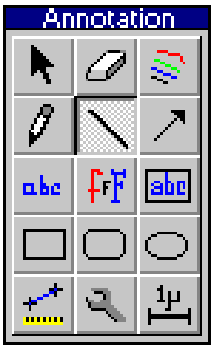
Select	Delete object	Change colours
Draw line	Straight line	Arrow line
Text	Change font	Multi-line text
Rectangle	Rounded rectangle	Ellipse
Distance freehand line	Annotation setup	Calibration scale

Adding an Annotation

The **Annotation Toolbox** allows you to add pointers, text and calibration markers to the image. Use annotation to draw attention to and provide further information on interesting features within the image. The annotation is saved as a separate layer of information, and does not alter the image itself, but can be saved with the image. Separate annotations are maintained for each image window.

Refer to: *Chapter 7* for more information on the image toolbar.

To open the **Annotation Box** click the **Annotation** button in the image toolbar to display the **Annotation** toolbox.

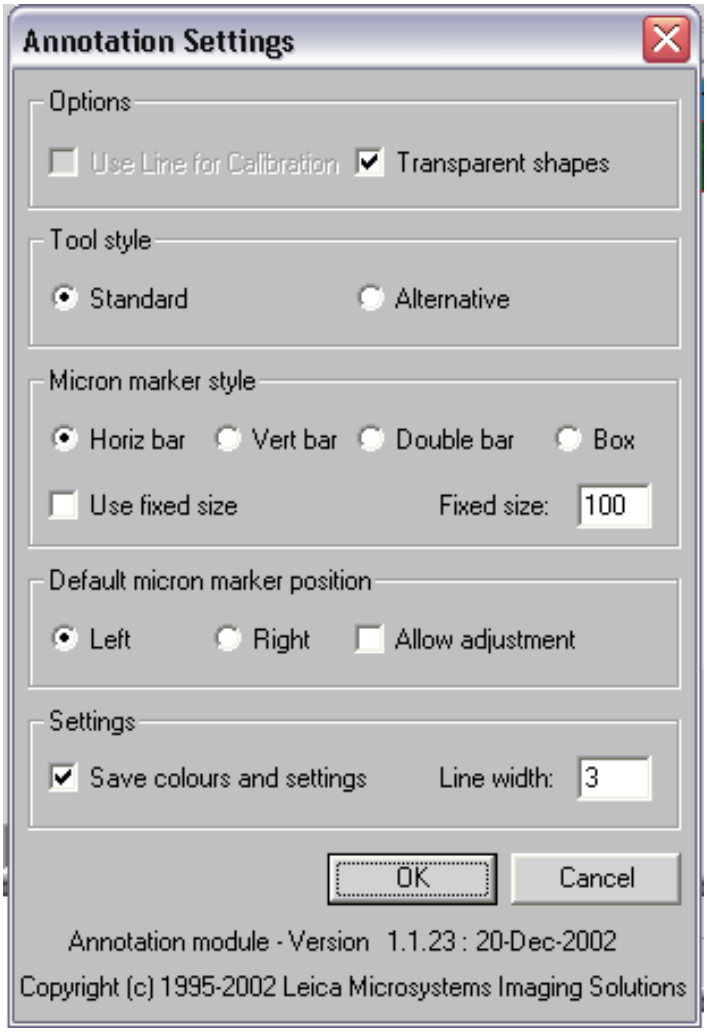


➡ To switch off displaying the **Annotation Toolbox**, click **Annotation** in the **Image Toolbar**.

Changing the Annotation Toolbox Settings

You can change the settings on your Annotation Toolbox by using the **Annotation Settings** dialog.

➡ To change annotation toolbox settings click the **Annotation Setup** button in the **Annotation Toolbox**.



To customise the annotation toolbox:

1. Choose from two options:
   - Check **Use Line for Calibration** if you want to use the **Draw Line** button for calibrating the system. This is not usually necessary.
   - Check **Transparent Shapes** if you want to display only the outlines of shapes. If this box is unchecked, shapes will appear filled in.
2. Select a style for the toolbox.
3. Choose from either **Standard** or **Alternative**.

Note: If you want to measure an angle in the image, you will need to select the **Alternative** toolbox style.

4. Specify a style for the micron marker. Choose from:

Horizontal bar **Vertical bar** **Double bar** **Box**

5. Specify whether you want the micron marker to appear on the **left** or the **right** of the image.
6. Specify the **line width**, in pixels.
7. Check **Save Colours and Settings** if you want to retain your settings after you exit **Leica FW4000**.
8. Click **OK** to apply the settings and close the dialog or **Cancel** to close the dialog without saving the settings.

Displaying Annotations

- To display annotations, click with the right mouse button in the main image window or a gallery image and select **Display Annotation** from the right-click menu.
- To view annotations ensure that the **Display Annotations** box is checked.
- Alternatively, you can use the keyboard shortcut **Ctrl+D** to display annotations.

Clearing Annotations

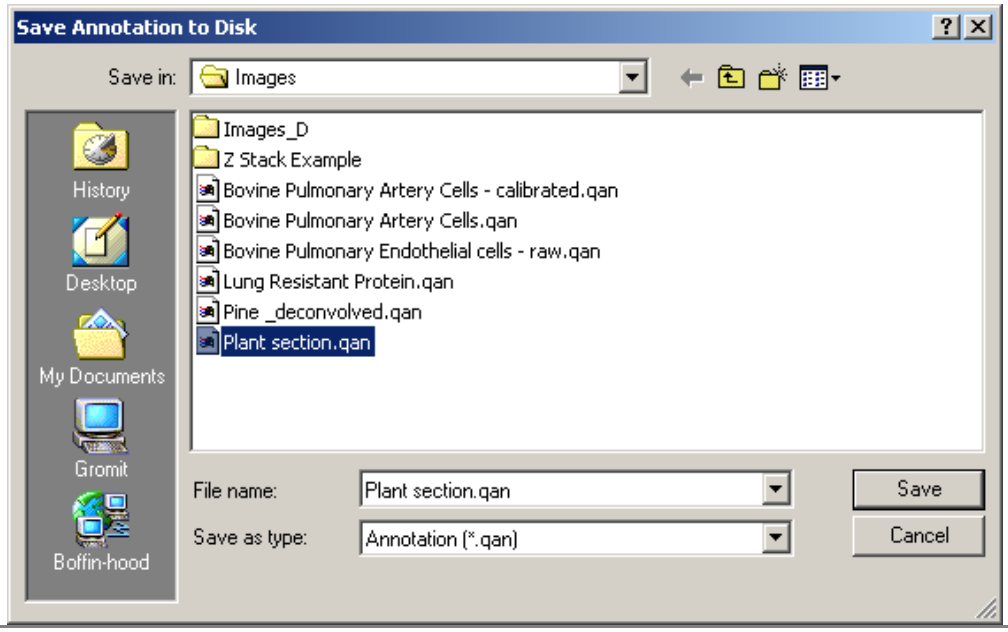
To remove an annotation:

1. Open the **View** menu.
2. Select **Clear Annotation**.

Saving Annotation

To save an annotation overlay to disk:

1. Open the **View** menu.
2. Click **Save Annotation**.



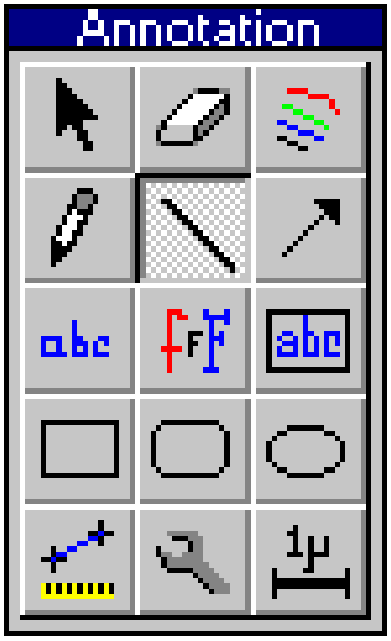
Note: Annotations are automatically saved when you save an image. However, it is useful to be able to save and load specific annotation files. You might, for example, use an annotation file as a template. This might contain the name and address of your laboratory or other information that is required to appear frequently. You can then include this information on an image without having to type it again.

Measuring an Angle

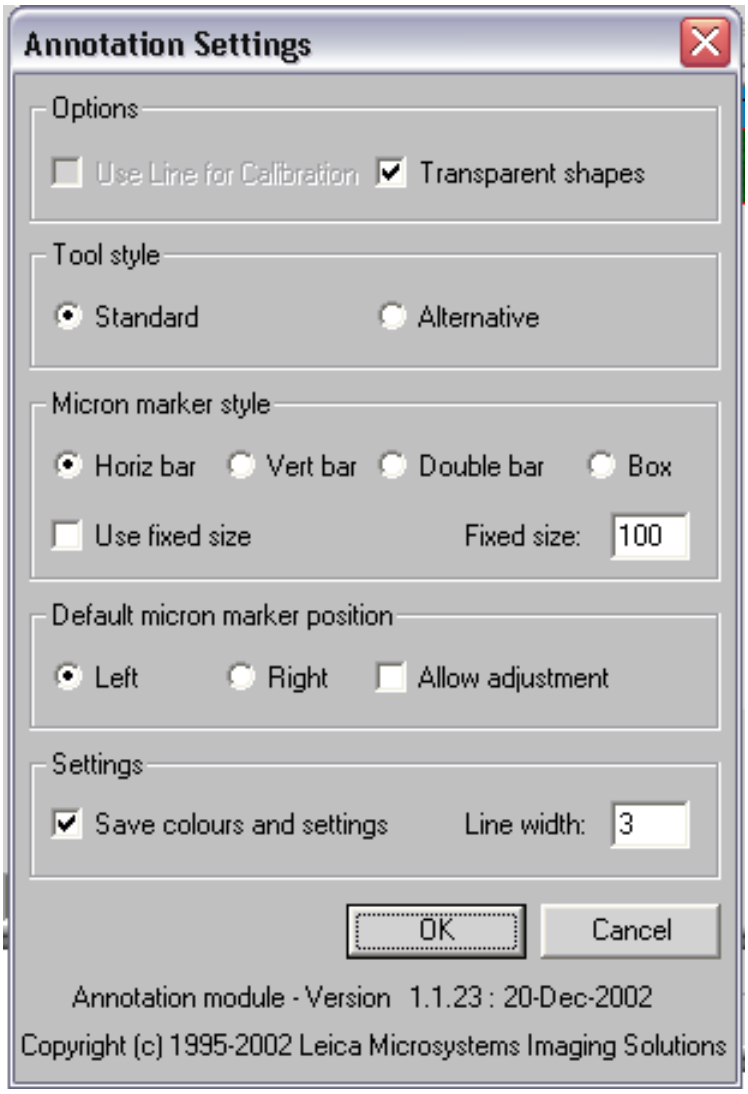
If you want to measure an angle, you will need to change the style of the Annotation Toolbox.

To change the style of an Annotation box:

1. Click the **Annotation** button in the **Image** toolbar to display the Annotation Toolbox.



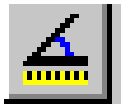
2. In the Annotation Toolbox, click the **Annotation Setup** button to open the **Annotation Settings** dialog.



3. In the **Tool style** section of the dialog, select **Alternative**. This changes the style of the toolbar, adding the **Measure Angle** button.

To measure the angle between two lines:

1. Select the **Measure Angle** button.



2. Draw the angle on the image by clicking at the start of the first line.
3. Click at the vertex.
4. Click at the end of the second line.
5. The angle will be displayed on the image as shown on the right side.

Printing a Set of Results

➡ To print a set of results, click the **Print** button in the main toolbar in the **Measurements** dialog.

Chapter Nine: Enhancing your Image

Chapter Overview

This chapter contains the following topics:

- Opening the Image Viewer
- Removing the Background
- Maximum Projection
- Channel Mixing
- Sharpening an Image
- Smoothing an Image
- Enhancing the Contrast of an Image
  - Image Equalisation
- Aligning the Component Images
- Converting an Image to Brightfield

Opening the Image Viewer

Before you start to process images in the **Image Viewer**, you must select and export a component image, as required, from the Image Gallery.

You can open the Image Viewer by either:

- Double-clicking on the image in question in the gallery.
- Click **Process** in the Experimental Setup.

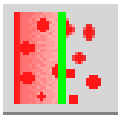
Note: Before you export a set of images for processing, ensure that you have selected a sensible set of images.

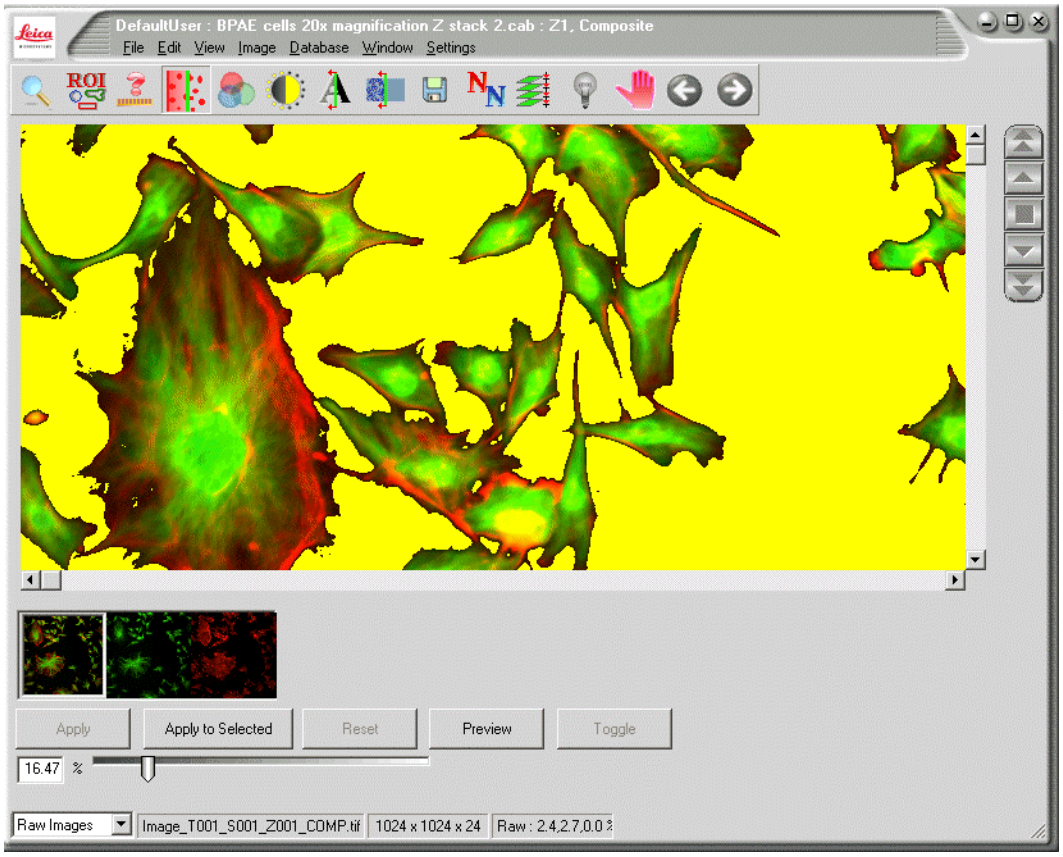
Refer to: *Chapter 6, Preparing Selected Images for Processing* for further information.

Removing the Background

To remove the background from the image:

1. Open the **Image Viewer**.
2. Select the **Image** menu and click **Background Removal**.
3. Alternatively, click **Remove Background** in the **Image Toolbar**.

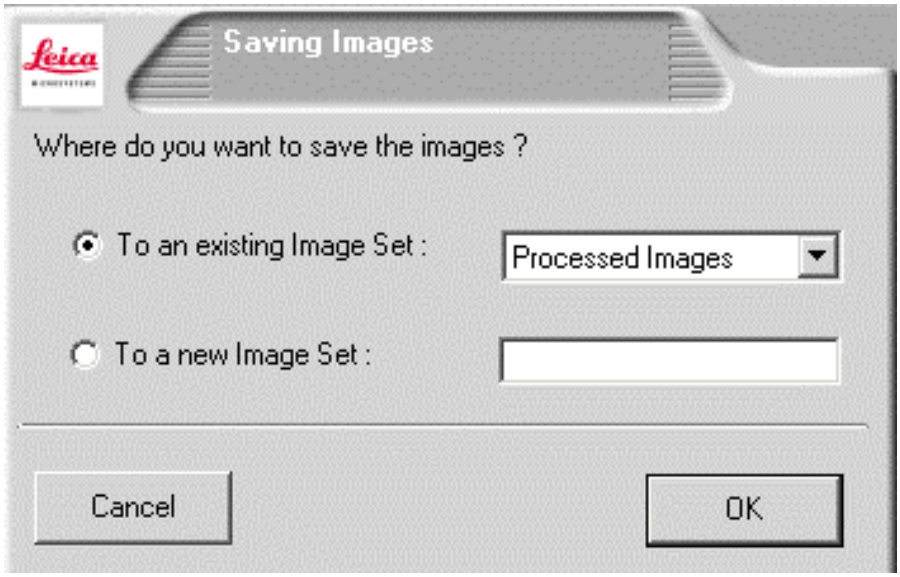




4. To adjust the threshold level move the slider from left to right. You will see the image change as you make this adjustment.

➡ Alternatively, you can type the threshold value you require into the % box to the left of the threshold slider.

5. Click **Preview** to preview the changes without making any permanent alterations.
6. Click and hold down **Toggle** to view the original image. Using this button you can compare the two versions of the image before deciding whether or not to retain your changes.
7. Click **Apply** or **Apply to Selected** in the **Image Viewer** to save your enhanced processed images. The **Saving Images** dialog allows you to define the processed image folder or the defined image set as the location for saving your images.
8. Click **OK** to save your processed image or stack, or click **Cancel** to close the dialog without saving any changes.



Refer to: *Chapter 7, Using a Processed Image Set* for further information.

WARNING: When using the **Apply to Selected** function, ensure you have selected the image set you wish to process in the gallery.

Maximum Projection

Note: **Maximum Projection** is a **Leica FW4000** product option that is only available if you have purchased the Enhancements module along with **FW4000Z** or **FW4000TZ** modules.

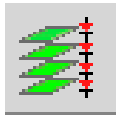
Maximum projection takes a Z-stack and selects the highest intensity pixel in the Z-plane for an X/Y position.

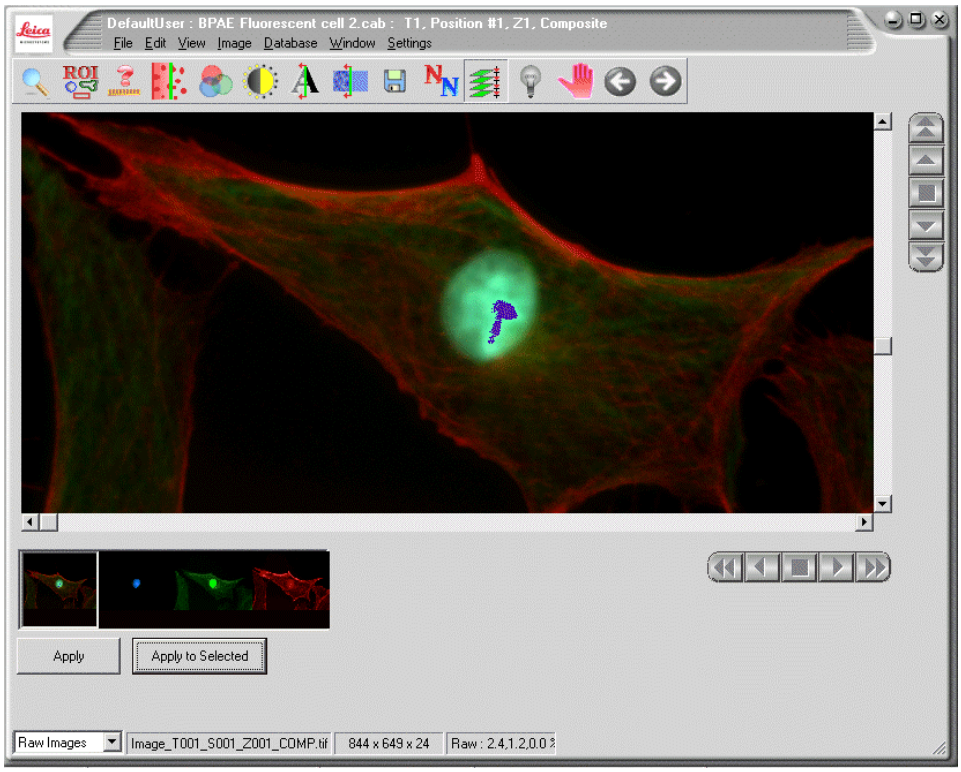
The max projection you created is stored as a raw image. If you perform another operation and save the image it will be a processed image.

To set the maximum projection:

1. Open the Image Viewer.
2. Select the **Image** menu and click Max Projection to display the Image Viewer as shown.

Alternatively, to set max projection, click the **Max Projection** button in the **Image Toolbar**, and display the **Image Viewer** as shown.





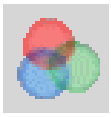
3. Click **Apply** or **Apply to Selected** in the **Image Viewer** to save your enhanced processed images.
4. Click **OK** to save your processed image or stack, or click **Cancel** to close the dialog without saving any changes.

Channel Mixing

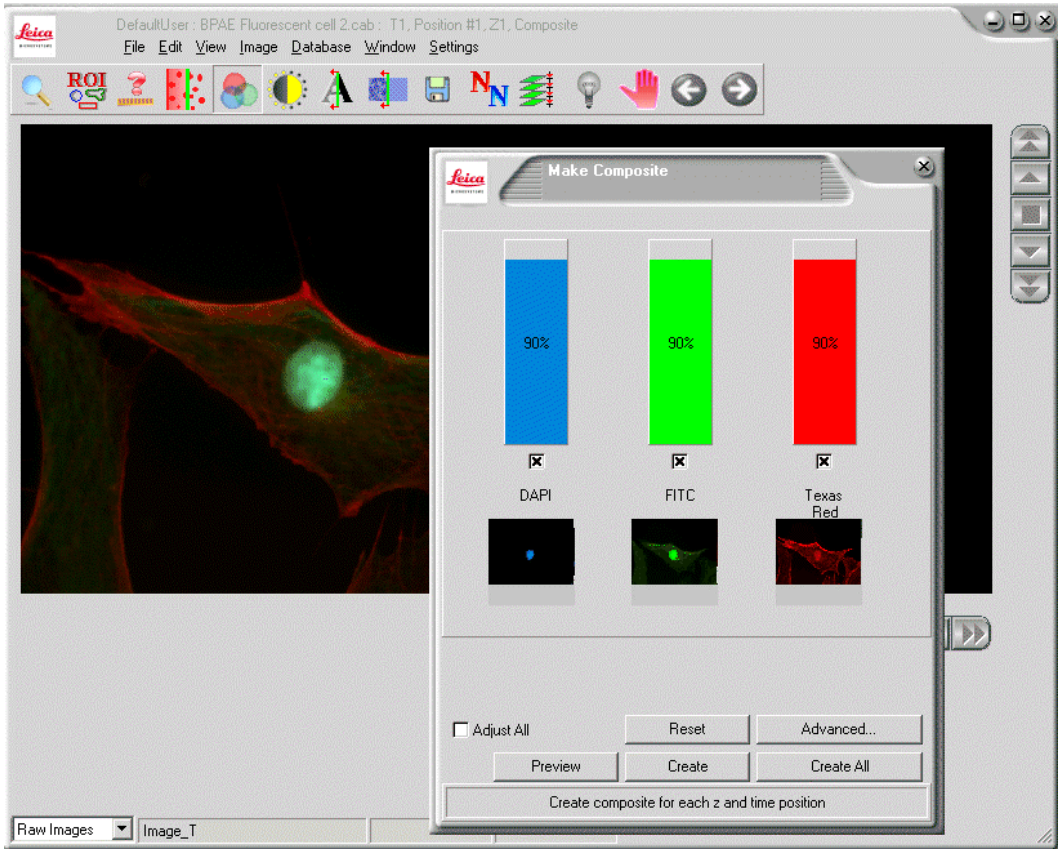
Channel mixing allows you to adjust the colour intensity for each probe.

To adjust the colour intensity of each probe:

1. Open the **Image Viewer**.
2. Click the **Make Composite** button from the **Image Toolbar**. The **Channel Mixing** dialog is displayed.



➡ Alternatively, click **Image** in the **Image Viewer** main menu and choose **Composite** to display the **Channel Mixing** dialog.



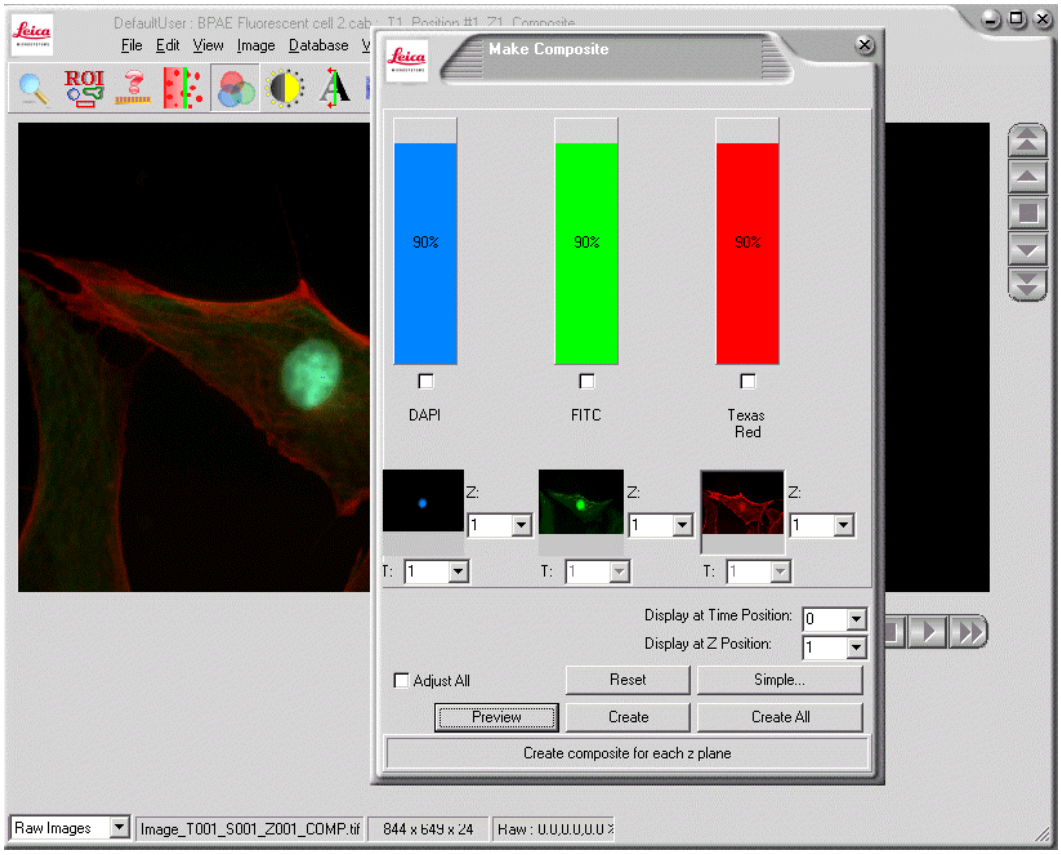
➡ To change the intensity of a probe, hold the left mouse button down on the coloured bar and drag it up and down, or click at the new level required.

➡ To adjust all probe intensities simultaneously, check **Adjust All**.

➡ Click **Reset** to reset the intensities to their previous values.

➡ To apply changes, click **Create** or **Create all** in the Image Viewer to close the Channel Mixing dialog and apply the changes. You can close the Channel Mixing dialog without saving any changes by clicking the cross in the top right corner of the dialog.

➡ If your experiment comprises Z-stacks and/or Time Sequences you can make composite images from different Z positions in the stack, and from different time sequences by clicking **Advanced**. When selecting values of Z or T for compositing, the systems displays all available values from your experiment in the scroll menu.



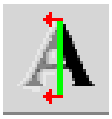
➡ To define where the new composite image should be placed in the Z-stack, click **Display of Z position**.

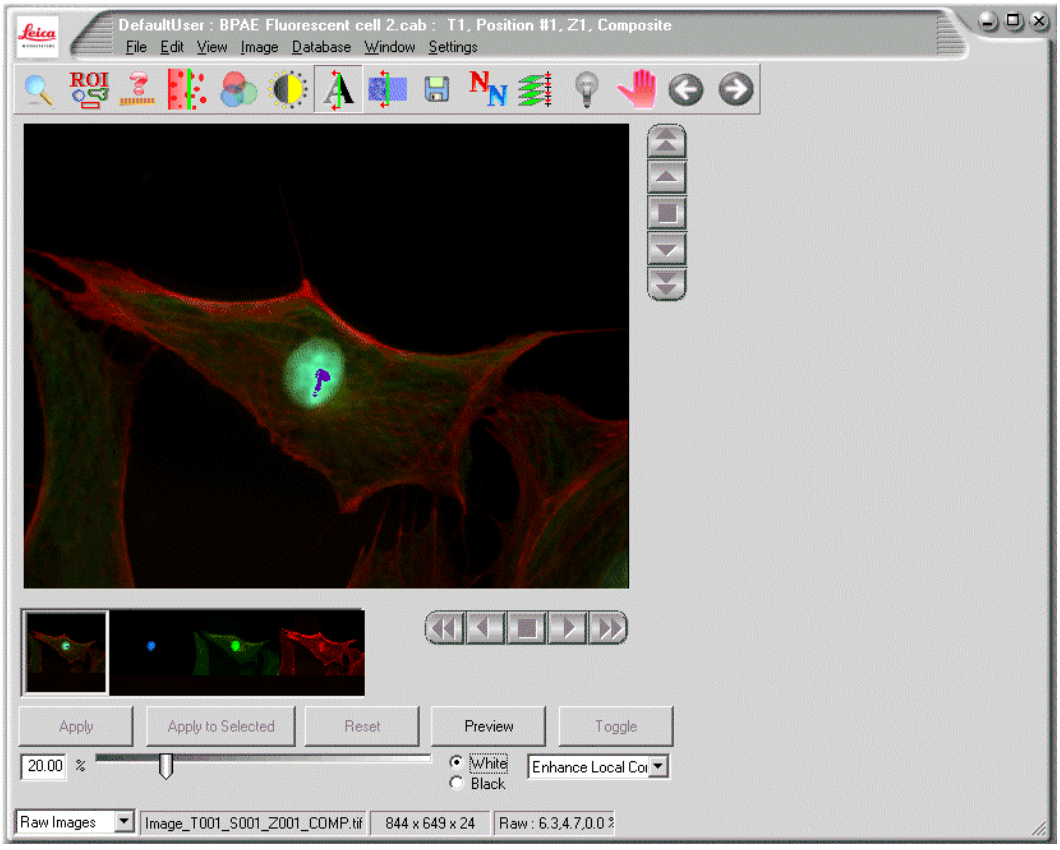
Sharpening an Image

To sharpen an image:

1. Open the Image Viewer.
2. Select **Image** from the Image Viewer main menu and click Sharpen to display the controls for sharpening an image.

➡ Alternatively, click **Sharpen** in the **Image Toolbar**, to display the controls for Sharpening the selected image.





To select an image to be sharpened:

1. Select **White** to sharpen lighter features in the image.
2. Select **Black** to sharpen darker features in the image.

➡ To select a sharpening amount move the slider bar to adjust the sharpening amount. The larger the value, the more severe the effect.

To choose between different sharpening operations select an **Operation** from the drop-down list. Available operations are:

Enhance local contrast Improves the contrast of small details in the image, while generally retaining the overall contrast of the image. Use the **Amount** slider to set the size of detail you are targeting for enhancement.

Extract small details Pulls small features out of the image, up to the size specified by the **Amount** slider. Only those details that are smaller than the specified size will be present in the resulting image. Using small amounts (3 to 10, for example) is an effective way of picking out sharp details in a larger area of fluorescence, while larger amounts (11 to 20) are useful for removing areas of uneven background fluorescence.

Tip: The resulting image may not have as much contrast as the original image. To correct this, try an **Auto-Stretch Intensity** operation, accessible in the **Contrast** tab.

The following table gives some suggestions on which operation to use:

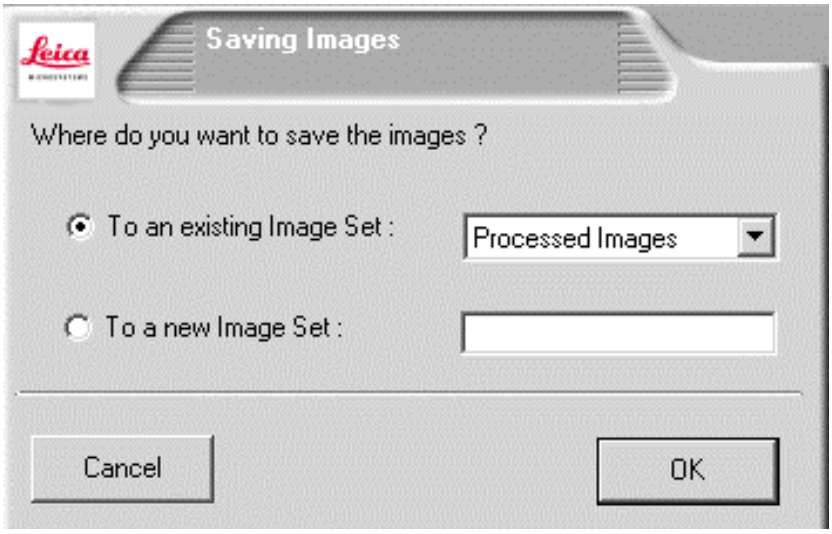
To…	Try…	Type	Amount
Enhance banding patterns	Enhance local contrast	Black	2 to 6
Extract small probe signals	Extract small details	White	1 to 8
Correct uneven background shading	Extract small details	White	8 to 20

To accept the selected sharpening enhancements:

1. Click **Preview** to preview the changes without making any permanent alterations.
2. Click and hold down **Toggle** to view the original image. Using this button you can compare the two versions of the image before deciding whether or not to retain your changes.
3. Click **Apply** or **Apply to Selected** in the **Image Viewer** to save

your enhanced processed images. The **Saving Images** dialog allows you to define the processed image folder or the defined image set as the location for saving your images.

4. Click **OK** to save your processed image or stack, or click **Cancel** to close the dialog without saving any changes.



Refer to: *Chapter 7, Using a Processed Image Set* for further information.

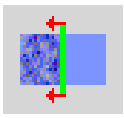
5. To sharpen only a defined part of the image, open the Image Viewer **Settings** menu and click **Use ROI**.

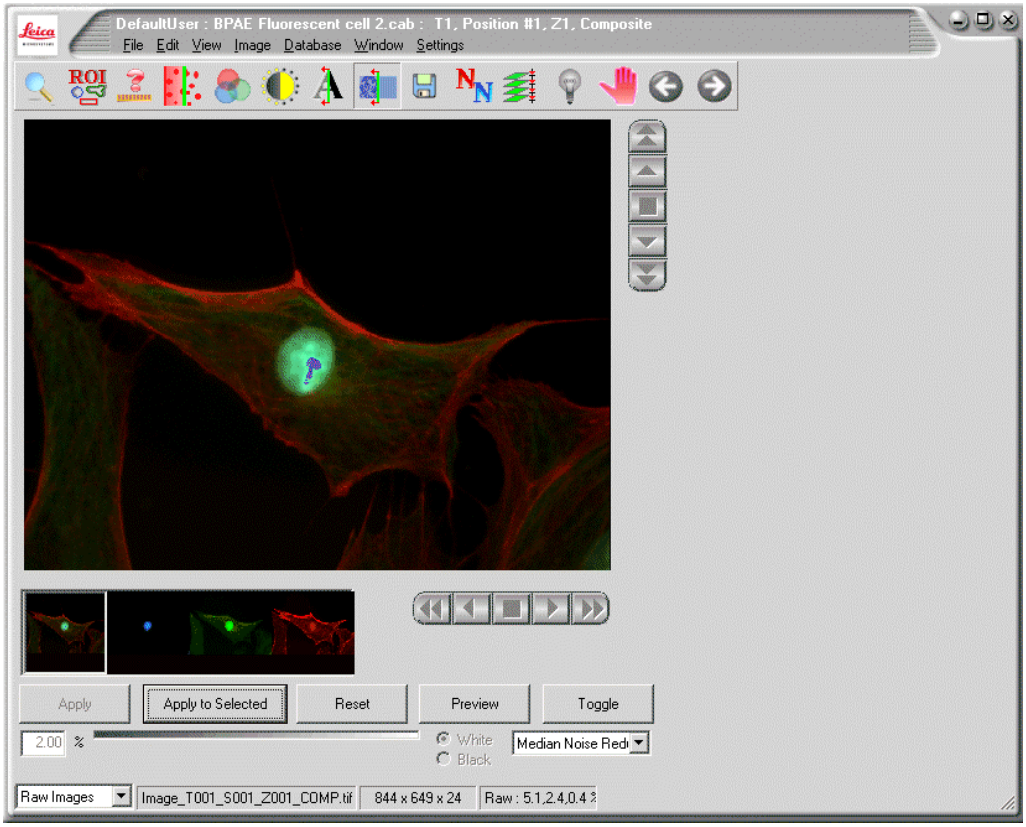
Smoothing an Image

To smooth an image:

1. Open the **Image Viewer**.
2. Select the **Image** menu and click **Smooth** to display the controls for smoothing an image.

➡ Alternatively, click **Smooth** in the **Image Toolbar** to display the controls for smoothing an image.





By removing noise from an image through smoothing, you can sometimes improve the effect that other subsequent image processing steps will have. For example, the **Auto-Contrast** enhancement in the **Image Contrast** dialog often gives a better result for very noisy images if performed after a smoothing operation, such as **Median Noise Reduction**.

To select a smoothing type:

1. Select **White** to smooth lighter features in the image.
2. Select **Black** to smooth darker features in the image.

➡ To select a smoothing amount, move the slider to set the amount of smoothing. The larger the value, the more severe the effect.

➡ To choose between different smoothing operations, choose an **Operation** from the drop-down list. Available operations are:

Median Noise Reduction	Removes noise that is one or two pixels in size without drastically affecting the sharpness of larger features.
Morphological Fillholes	Fills in small features up to the size specified by the **Amount** slider. Details that are smaller than the size specified will be removed in the resulting image, while the sharpness of the edges of larger features is kept intact.
Matrix Averaging	Blurs the image. The amount of blurring is set by the **Amount** slider, and affects all features in the image.
Weighted Averaging	This is an alternative form of the matrix averaging that gives a slightly softer smoothing, preserving more of the image information for a given amount.
Spot Removal	This option allows you to remove spots from an image once it has been acquired. The **Amount** setting controls the spot removal factor. This defines how bright (in relation to the surrounding pixels) the spot must be before it is removed. In general, the lower the amount the more spots will be removed. A typical value would be **20**.

The following table gives some suggestions on which operation to use when:

To…	Try…	Type	Amount
Remove noise	Median Noise Reduction	N/A	1 to 2
Noise removal (alternative)	Weighted Averaging	N/A	1 to 3
Remove small features and maintain hard edges for larger features	Morphological Fillholes	White	1 to 5
Blur unwanted small detail	Matrix Averaging	N/A	1 to 5
Remove spots	Spot Removal	N/A	20

To accept the enhancements you have selected:

1. Click **Preview** to preview the changes without making any permanent alterations.
2. Click and hold down **Toggle** to view the original image. Using this button you can compare the two versions of the image before deciding whether or not to retain your changes.
3. Click **Apply** or **Apply to Selected** in the **Image Viewer** to save your enhanced processed images. The **Saving Images** dialog allows you to define the processed image folder or the defined image set as the location for saving your images.
4. Click **OK** to save your processed image or stack, or click **Cancel** to close the dialog without saving any changes.

Refer to: *Chapter 7, Using a Processed Image Set* for further information.

5. To sharpen only a defined part of the image, open the Image Viewer **Settings** menu and click **ROI**.

Enhancing the Contrast of an Image

Before an image is acquired, the brightness and contrast are determined by a number of factors:

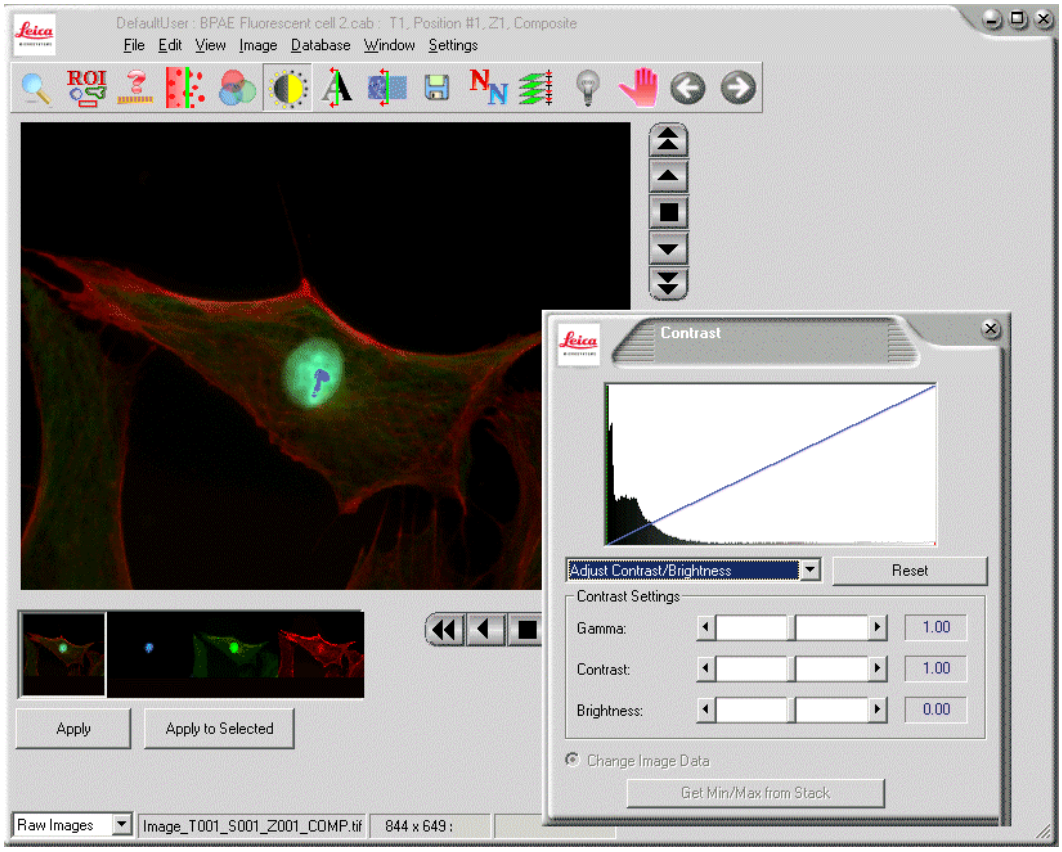
- Specimen signal intensity
- Microscope setup
- Camera settings and integration time
- Video levels

In some cases, even careful attention to these parameters does not provide a sufficiently high contrast image. In these cases, you can use the image enhancement capabilities to adjust the contrast and brightness of an image that has been acquired.

To enhance the contrast of an image:

1. Open the **Leica Image Viewer** dialog.
2. Select the **Image** menu and click **Contrast** to display the **Image Contrast** dialog.

Alternatively, click **Contrast** in the **Image toolbar** to display the **Image Contrast** dialog.



➡ To recover the original image when you have checked **Change Display Only**, click **Reset**.

➡ Use the **Gamma** slider to make the darker areas in the image brighter or the lighter areas darker.

Note: Gamma correction is a logarithmic transfer function.

- Gamma values less than 1 tend to brighten the darker parts of the image more than the brighter parts.
- Gamma values greater than 1 cause the darker parts to be darkened more than the brighter parts.
- A value of 1 is the default, it does not change the image.

➡ Use the **Contrast** slider to adjust the relative contrast difference between the light and dark features in the image. A value of 1.0 indicates that no adjustment is made. Values less than 1 decrease the contrast, while values greater than 1 increase the contrast.

➡ Use the **Brightness** slider to adjust the overall brightness of the image. A brightness setting of 0.0 indicates that no adjustment is made. Values less than 0 indicate a darkening of the image, while values greater than 0 indicate a lightening of the image.

➡ Alternatively, choose a contrast operation by clicking **Operation** from the drop-down list. Available operations are:

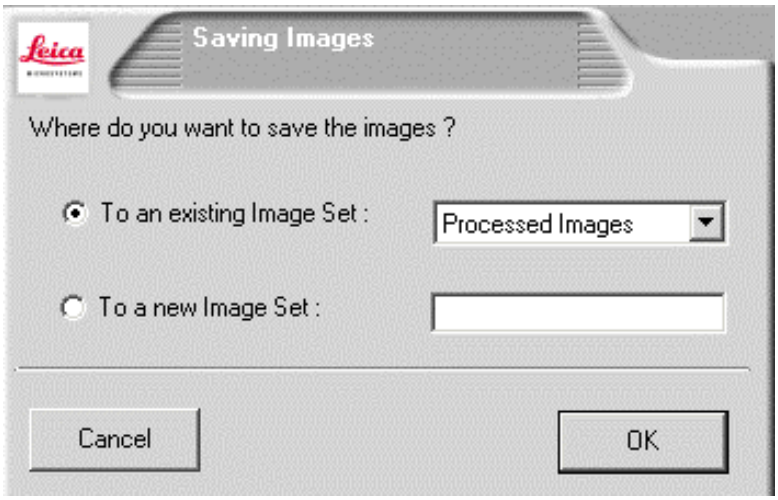
Adjust Contrast/ Brightness	Allows you to adjust the image contrast and brightness manually. Use the **Contrast** slider to adjust the relative contrast difference between the light and dark features in the image. Use the **Brightness** slider to adjust the overall brightness of the image. A brightness setting of 0.0 indicates that no adjustment is made.
Auto-stretch Intensities	Automatically determines the minimum and maximum intensities in the image, and adjusts the contrast and brightness as needed to ensure the image occupies the full sensitivity scale. This operation can be sensitive to noise, so it is sometimes useful to perform a smoothing operation first for a stronger effect.
Histogram Equalisation	Automatically evaluates the intensity distribution of the image and adjusts the contrast and brightness to give a more evenly weighted distribution.
Logarithmic Fit	Automatically adjusts the contrast curve to a logarithmic distribution, improving the visibility of dim features.
Exponential Fit	Automatically adjusts the contrast curve to an exponential distribution, reducing the visibility of dim features.
Square Root Fit	Automatically adjusts the contrast curve to a distribution based on the square root of the intensity, increasing the visibility of dim features.
Invert	Inverts the image.

Tip: In most cases the **Adjust Contrast/Brightness** option will give the best result. Try increasing the contrast level to increase the range of intensities, then decrease the brightness level to help suppress the lower background signals.

➡ To sharpen only a defined part of the image, open the **Image Viewer Settings** menu and click **ROI**.

To accept the contrast changes you have made:

1. Click **Apply** or **Apply to Selected** in the **Image Viewer** to save your enhanced processed images.
2. Click **OK** to save your processed image or stack, or click **Cancel** to close the dialog without saving any changes.



Refer to: *Chapter 7, Using a Processed Image Set* for further information.

Image Equalisation

You can set maximum and minimum pixel intensity for an entire sequence of selected images. Equalisation of the image intensities may be applied by clicking on the **Contrast** icon in the Image Viewer toolbar.

➡ To set the values for a stack, select only the stack in the Image Gallery and then use **Apply to Selected.**

➡ To set the values for a single image, click **Apply**.

- To set the values for a sequence of images that you have selected in the Image Gallery, set **Apply to Selected**.
- To take maximum and minimum values from a captured stack (that is, to take the maximum intensity pixel in the whole stack and the minimum pixel intensity in the whole stack), and to apply these to every image in the stack, click **Get Min/Max from Stack**, followed by **Apply to Selected**.

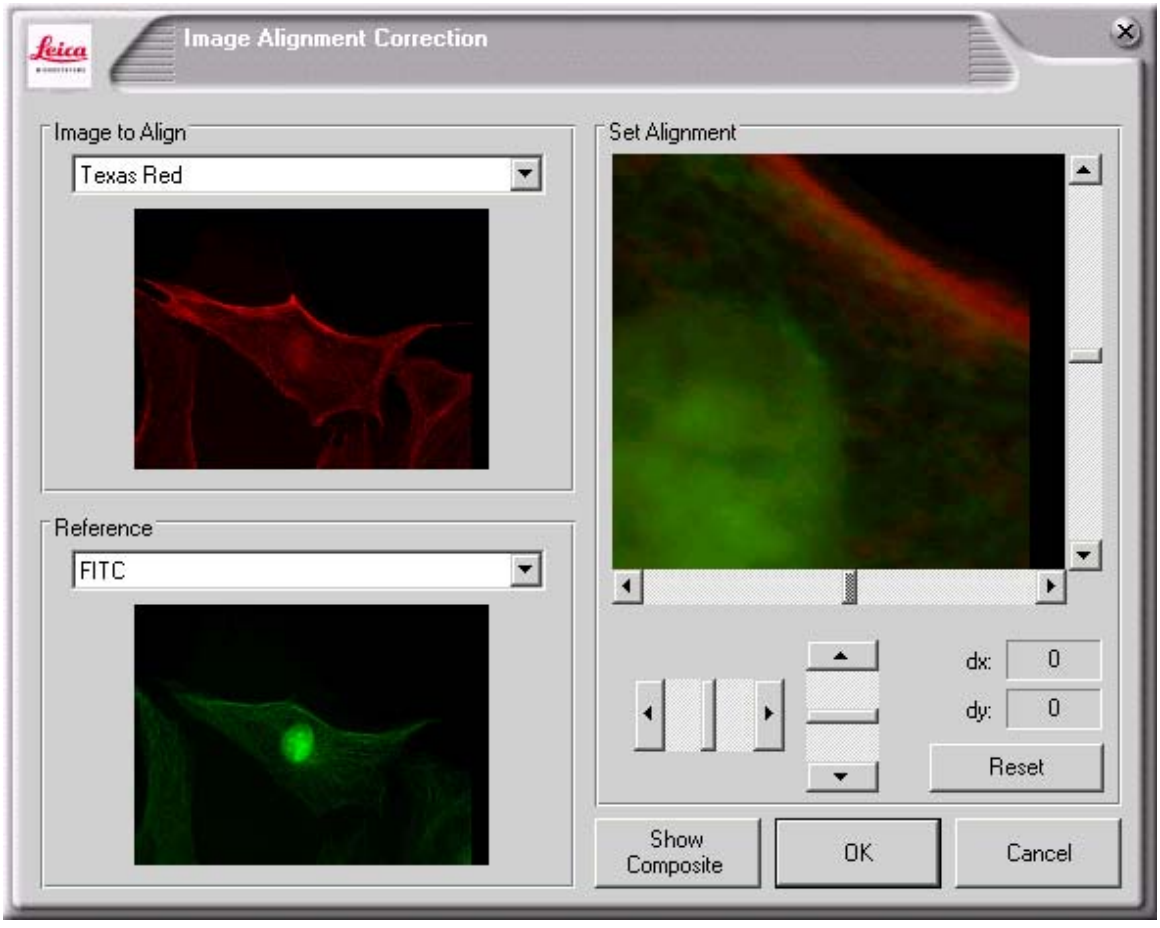
Aligning the Component Images

Note: This operation can only be performed on component images. However, you can select this option even when the composite image is selected. You do not have to choose a component image **before** opening the dialog.

To align the component image:

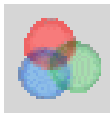
1. Open the **Image Viewer**.
2. Select the **Settings** menu and click **Alignment Correction** to display the **Image Alignment Correction** dialog.

➡ Alternatively, right click on the image window to display the **Right-Click Menu** and choose **Alignment Correction**.



➡ Choose the **Image to Align** and the **Reference** image from the drop-down lists.

- Select an area for alignment by dragging the mouse over the area you want to align on either the **Image to Align** or the **Reference** image. The selected area will be displayed in the **Set Alignment** area on the right side of the dialog.
- Use the slider bars to move the **Image to Align** in relation to the **Reference** image. The effect is displayed in the **Set Alignment** area of the dialog.
- Click **OK** when you are satisfied with the changes. To close the dialog without saving any changes, click **Cancel**. To reset the values to zero, click **Reset**.
- Before you can see the effect of the alignment, you must recombine the images. To do this, click the **Show Composite** button.

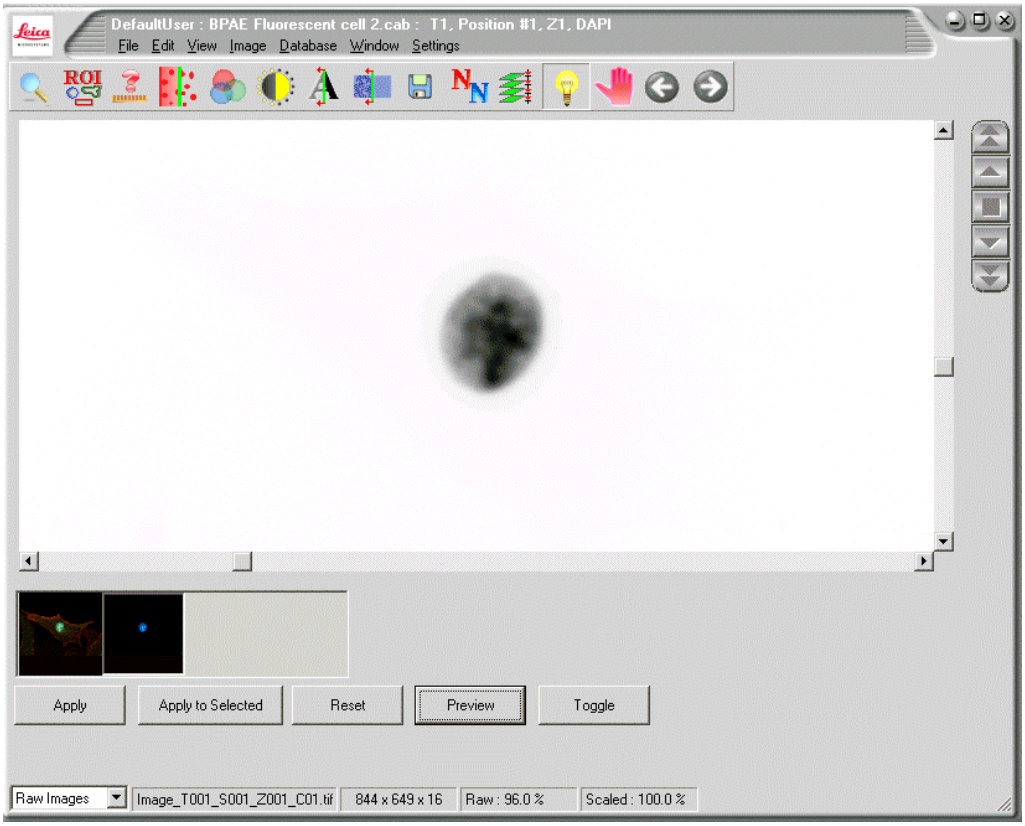


Converting an Image to Brightfield

Note: This option is only available for the **component** images.

To convert an image to brightfield:

1. Open the **Image Viewer**.
2. Select **Brightfield** from the **Image Toolbar**.

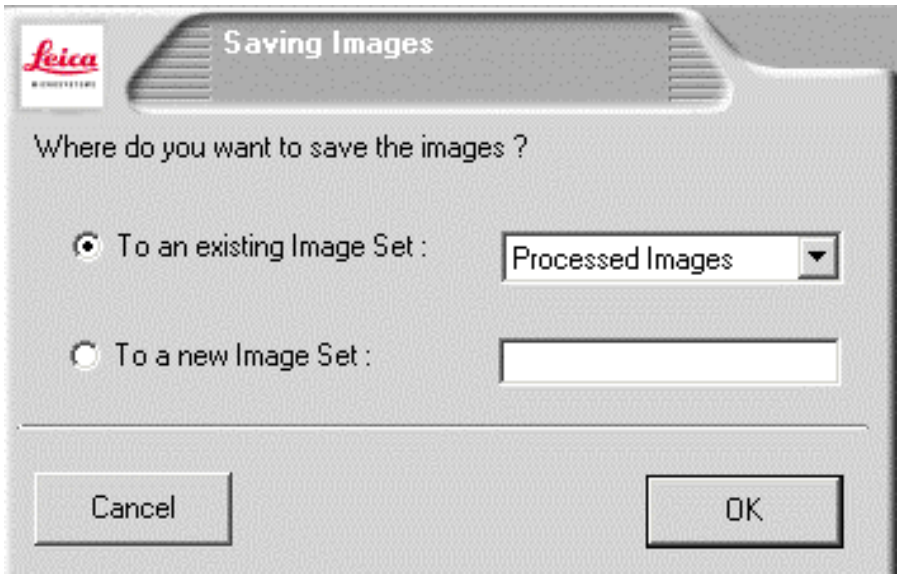


To accept the brightfield changes to your images:

1. Click **Preview** to preview the changes without making any permanent alterations.
2. Click and hold down **Toggle** to view the original image. Using this button you can compare the two versions of the image before deciding whether or not to retain your changes.
3. Click **Apply** or **Apply to Selected** in the **Image Viewer** to save your enhanced processed images. The **Saving Images** dialog

allows you to define the processed image folder or the defined image set as the location for saving your images.

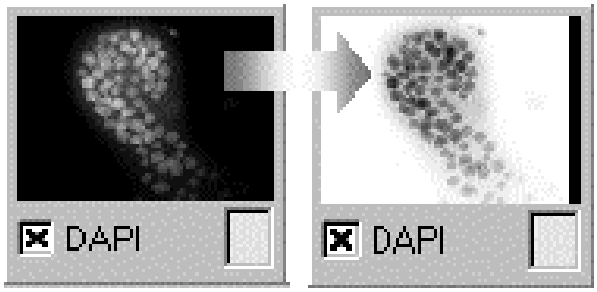
4. Click **OK** to save your processed image or stack, or click **Cancel** to close the dialog without saving any changes.



Refer to: *Chapter 7, Using a Processed Image Set* for further information.

This option can be used on fluorescent images that have a dark background and a light specimen. You can convert the image to brightfield by performing an inversion, resulting in a dark specimen on a light background.

The original image is shown below on the left, and the image after brightfield has been applied is shown on the right.



Chapter Ten: Advanced Deconvolution

Chapter Overview

This chapter contains the following topics:

- Exporting Images from Leica FW4000 to Leica Deblur and 3D Visualise
- Tutorials for the Deblur View Menu Items
  - Single View
  - Triple View
  - 3D Viewer
  - Enhancement
- Tutorials for Projections Menu Items
  - Maximum Projection
  - Minimum Projection
  - Sum Projection
  - Voxel
  - Slice Viewer
  - Alpha Blending
  - Best Focus
- Tutorials for Channels Menu Items
  - Red Channel
  - Green Channel
  - Blue Channel
  - All Channels
  - Viewing the Channels and Slices of a Multi-channel Data Set
- Tutorials for Settings Menu Items
  - Image Dimensions

- Spacing (Microns)
- Data Background
- Deconvolution Settings
- Standard Settings
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- Tutorials for Operations Menu Items
  - Crop
- Tutorials for Movie Menu Items
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  - Open a Movie
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- Tutorials for Start Deconvolution
  - Start 3D Blind Deconvolution
  - 2D Deconvolution
  - Inverse Filter
  - Nearest Neighbours/No Neighbours
  - Axial Blind Deconvolution
- Tutorial for Batch Menu Item
  - Batch Process

Exporting Images from Leica FW4000 to Leica Deblur and 3D Visualise

Note: This Chapter is applicable only to users of the **Leica Deblur and 3D Visualise** software.

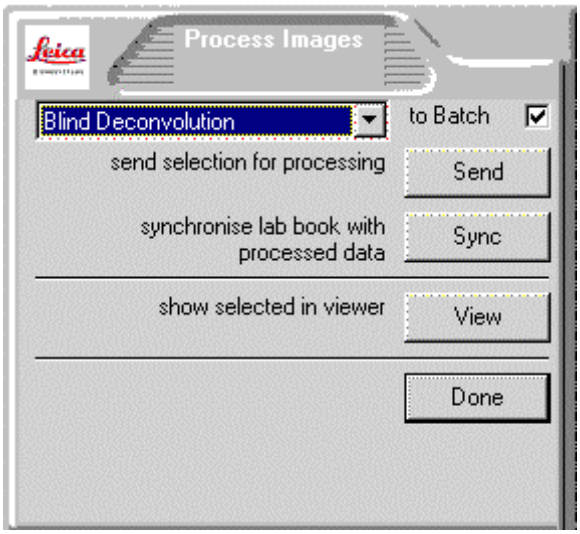
This chapter describes the steps involved in passing image sets from the **Leica FW4000** to the **Leica Deblur and 3D Visualise**.

Before you can process your images in **Leica Deblur and 3D Visualise** you must specifically select them and export them to the Image Viewer.

To prepare images for processing:

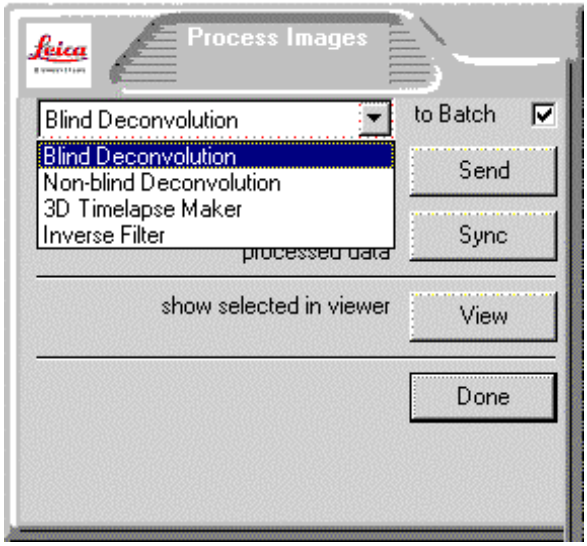
1. Open the Leica Image Gallery.
2. Select the individual images you want to process.
3. Click the Image Gallery **Gallery** menu and click **Export Selected Images to Image Processing**.
4. Close the **Leica Image Gallery**.

Click the process arrow in the Environmental Process flow to display the **Process Images** dialog.



To select the action to be taken on a set of images when exporting them to Leica Deblur:

1. Click to Batch in the **Process Images** dialog.
2. Open the drop-down menu adjacent to the Batch check box.



3. Select the required processing feature from the menu.
4. Press **Send** to send the selection for processing.

You may repeat steps 1 to 4 for each experiment from which you wish

to select images by opening that experiment. The Batch queue can store requests from many exeriments.

- Alternatively, you can press **View** to open the **Image Viewer** to use the process tools available.
- To return processed sets of images to the experiment folder in which it was created use the **Sync** button.

Note: After batch processing has completed, you must ensure that each experiment is opened and Sync'd for each set of images used for batch processing. Remember that deconvolution may have made some intermediate results as well – Sync will collect all of them for the current experiment.

Tutorials for the Deblur View Menu Items

Single View

This Deblur feature allows you to choose the view in which the data set is presented. The data can be viewed from three orthogonal perspectives. The choices available are: XY, ZY, and XZ where X represents the image width, Y the image height, and Z is the depth or may be thought of as the optical axis in microscopy applications. When a choice is made, the single view that is generated will be for the currently active projection. For example, if a Sum Projection is the active view, selecting ZY will generate a ZY Sum Projection of the view.

- XY is the view of the "face" of the data set or the front view of each slice.
- XZ is an "edge-like" view of the data set or of one of its slices. It can be thought of as a horizontal slice through the XY view.
- **ZY** is a "side" view of the data set or each slice. It can be thought of as a vertical slice through the XY view.

To view a vertical slice through the XY view:

1. With a suitable data set loaded, for example the **TLBview.tif data set**, select **XZ** from the **Single View** option under the **View** menu.

Note: Transmitted Light Brightfield data will open in the Min Projection view.

2. Set the **XY Min Projection** as the active view by selecting **TLBview.tif: XY Min Projection** (MULTI-CHANNEL) from the Windows menu item or by clicking within the XY Min Projection view.

Note: The status bar at the bottom of the main window will display the active view and projection.

Triple View

Selecting the Deblur Triple View feature allows you to create three orthogonal views of your data set. Each view is the Minimum Projection of the data. On the top left of the Triple View display is the XY view, on the top right is the ZY view, and on the bottom left is the XZ view of the data set. The TLBview.tif:XY Minimum Projection from the previous section should be active.

To activate the TLBview.tif:XY Min Projection:

1. Select **Triple View** from the **View** menu. The XY, ZY, and XZ views of the TLBview.tif data set are displayed in the Triple View box.
2. Make active the TLBview.tif: XY Min Projection data set.
3. From the **VISUALIZATIONS** menu select Slice Viewer. The data set will be displayed in a **Slice Viewer**.
4. From the **VIEW** menu, select **Triple View**. The XY, ZY, and XZ Optical Slice Viewers are displayed in the **Triple View** box. The red lines indicate where each slice is in relative position to the others in the data set.
5. To remove the red lines click **Hide Crosshair**.
6. To view the red lines click **Show Crosshair**.
7. To close the Triple View click on the **[X]** in the upper right corner of the **Triple View** box.
8. Close all data sets before continuing on to the next section.

3D Viewer

The 3D Viewer, accessed from the VISUALIZATIONS menu, displays a graphical presentation of the data set. This feature allows you to create oblique slices, orthogonal slices, movies, apply different colour maps, change projections, adjust subvolume size, rotate the data, and to view data in the anaglyph stereo mode.

To create graphical representations in the 3D Viewer:

1. With a suitable data set loaded, navigate to the SmallHip folder, open the SmallHip.avz data set.
2. Select the 3D V**iewer** from the **View** menu. The 3D Viewer window opens with your data set in the Orthogonal Slices view of its XYMax projection. In the upper left corner of the 3D Viewer window the red line represents the X-axis, the green line represents the Y-axis, and the blue line represents the Z-axis of the data set.

Note: The 3D Viewer has several menus items, for example: View, Projection, Axis, Oblique, Movie, Colour Map, Save, and Options. These menu items may also be accessed through the Control Panel found under Options. From the Axis menu select **Go to View > XZ**. The data set will be rotated to the XZ Orthogonal Slice view.

3. To rotate your data set: Click anywhere in the 3D Viewer window.
   - Hold down the left mouse button and move your mouse. The data set will rotate in the same direction your mouse moves.
   - Stop your mouse movement with the left button still depressed, to stop the rotation.
   - If your mouse is moving while you release the left mouse button, the data set will continue rotating.
   - Click in the 3D Viewer window to stop the auto-rotation.
4. Select **Cube Surface** from the **View** menu. From the **Oblique** menu select **Display Slice**. The Oblique Slice appears on the XY plane. To move the Slice hold down the Shift key, left click on

the slice and move your mouse.

- To crop the volume or move an Orthogonal/Oblique Slice hold down the Ctrl key, left click on the plane/slice and drag.
- To rotate the Oblique Slice hold down the Shift key, left click on the Oblique Slice and drag.
- To zoom the displayed image, right-click on the image and drag from bottom right to top left (or vice-versa).

Enhancement

Depending on the display of the machine which is running the software, images may appear too dark, or in other cases, too bright. To compensate for these differences you can perform a Gamma correction on a particular view.

To perform a gamma enhancement:

1. With a suitable data set loaded select **Image Enhancement** from the **View** menu. The **Image Enhancement** dialog appears.
2. Type a value of **1.2** in the **Gamma** field. You will notice the change in the image within the Image Enhancement dialog.

Note: A Gamma value of 1.0 restores the image to its original state. Values greater than 1 brighten the image, whereas values below 1 darken the image. Gamma values may be between 0.1 and 10.0.

3. You may also adjust the image **Black Level** and **Brightness** by using the scroll bar next to these adjusters.

Note: The Brightness and the Black Level values are used in a histogram stretching intensity filter.

For 8-bit data, this filter sets all the pixel values that are greater than the Brightness parameter to 255, and all the pixel values less than the Darkness parameter to 0. Pixel values, which fall in between the brightest and darkest parameter values, are linearly scaled between 0 and 255.

Image Enhancement may be done on a single colour channel (Red, Green, or Blue) or on All Channels by activating the appropriate choice under

Channel.

4. To return to the original data, click Cancel on the **Image Enhancement** dialog.
5. Click **OK** to close the **Image Enhancement** dialog.

Note: When applying Enhancement to a Slice Viewer projection, it is possible to change only the slice being displayed.

Note: When viewing in a Slice Viewer, the maximum and minimum values for Brightness and Black Level are the true maximum and minimum values of the entire data set. For other projections, the Brightness and Black Level values represent the maximum and minimum intensity values of the view.

Tutorials for Projections Menu Items

Maximum Projection

This function takes parallel rays, perpendicular to the viewing surface, and cast them through the image. The maximum voxel value encountered along each ray is taken for the projection pixel value, and the resulting image is made up of each maximum voxel value. This projection highlights edges and prominent bright features.

To use maximum projection:

1. With a suitable data set is loaded in the XY Maximum projection view select **XZ** from the **Single View** option under the **VIEW** menu. Choosing different views will display the image from different perspectives.
2. Set **XY Max Projection** as the active view by left-clicking in the view.

Note: The XY Max Projection view is the standard default view, except for transmitted light brightfield data, which opens in the XY Minimum projection view.

Note: The status bar on the lower left corner of the main window will display the active view and projection.

Minimum Projection

This function takes parallel rays, perpendicular to the viewing surface, and cast them through the image. The minimum voxel value encountered along each ray is taken for the projection pixel value and the resulting image is made up of each minimum voxel value.

The Minimum projection provides volumetric representations in which foreground intensities tend to be suppressed. This projection highlights edges and prominent dim features.

➡ To use minimum projection select **Minimum Projection** from the **Projections** menu. The Minimum Projection will be automatically calculated and displayed.

Sum Projection

This function takes all the voxel values along each parallel ray, perpendicular to the viewing surface, and sums their intensity values. This creates a projection of all the summed values. Sum Projections provide volumetric representations of the data in which more information from the data is considered than with the Maximum projection. Background noise will also be included in this type of projection.

➡ To generate a Sum Projection for your data set, either click on **Sum Projection** or select **Sum Projection** from the **Projections** menu.

Voxel Gradient

This function finds the first voxel that is above the intensity threshold for each parallel ray, perpendicular to the viewing surface, then computes the dot product of the gradient at this point. These dot product values make up the pixel intensity values in the 2D projection. This function is ideal for examining the surfaces of objects.

To show the Voxel Gradient:

1. Right Click on the XZ Max projection view of your data set and select **3D Viewer** from the menu
2. From the **Projection** menu, select **Voxel Gradient**

Note: The Voxel projection creates a shaded iso-surface for a volume at a specific threshold value.

Note: The default threshold for the minimum percentage box of the Voxel Gradient shading is 20%. This threshold can be set on the Control Panel of the 3D Viewer – under the Options Menu - or in the Operation Setting from the main File menu in Leica Deblur.

Slice Viewer

This function generates slices through the image "optically" and then displays the "face" of each slice in the Slice Viewer. The thickness of each slice corresponds to the Z spacing or step size. Viewing in the Slice Viewer is a good way of examining the degree of optical sectioning in the data. To control which slice is currently displayed, move the slider at the bottom of the view window. The current slice number is displayed to the left of the slider.

Tip: You are advised to examine your data in a Slice Viewer because it allows you to see subtle and not so subtle changes from slice to slice.

To use the slice viewer:

1. Make the XY Max Projection of your data set active by clicking within its view.
2. Select **Slice Viewer** from the **VISUALIZATIONS** menu to generate an optical slices projection of the data set from the XY perspective.

Note: When a projection operation is selected, the view formed depends upon the currently active view. For example, in the previous step the XY Max projection was the active view. Thus, when the Slice Viewer menu item was selected, optical slices were created from an XY perspective.

3. To step through the optical slices, move the slider at the bottom of the view. You can do this by clicking the slider and dragging it to the left or right, or by pressing either the left or right arrow key. The current slice number is displayed to the left of the AUTO button.
4. Press the **AUTO** button to the left of the slider to cause the Slice Viewer to cycle through all the slices automatically. AutoPlay displays the slices sequentially.
5. Once you have viewed all the slices, pressing the **STOP** button to halt AutoPlay.

Note: It is important you review any data set you would like to enhance by deconvolution. You are looking for a smooth transition of data between slices. A data set that is a good candidate for deconvolution will show the

slices slowly going into focus and then out of focus.

6. Close the **Slice Viewer** dialog clicking the close **x** in the upper right corner of the Slice Viewer dialog.

Alpha Blending

This function is available only in the 3D Viewer menu, and it simulates the effect of transparency, translucency and opacity in the data set. The alpha blending factor (percentage) defines by how much the intensity value of the reflected ray is modified after the ray reaches the first threshold value Voxel and passes through the sample on to the next threshold value Voxel. The Alpha Blending factor determines how the layers of the volume are displayed.

To apply Alpha Blending:

1. Make the Max Projection of your data set the active view.
2. Open the 3D Viewer from the **View** menu.
3. From the **Projections** menu select **Alpha Blending**. The Alpha Blending projection will be displayed in the window. Place the 2 views alongside each other to compare the views.
4. Click **Apply** to view the new Alpha projection.
5. Place the Alpha Blending Projection next to the Voxel Gradient Projection and compare the differences in their appearance.

Best Focus

This function takes parallel rays, perpendicular to the viewing surface, and cast them through the 3D image. The voxel, encountered along each ray, with the greatest local contrast is selected for display in the resulting 2D image. This produces an image constructed from the most in focus features from the volume.

A Best Focus projection creates an image out of the regions within a volume that best stands out against their neighbours. This is useful for observing more subtle features in an image, which may be not as pronounced in other projections.

To obtain the best projection:

1. Make the Max Projection of your data set the active view.
2. Select **Best Focus** from the **VISUALIZATIONS** menu. **Leica Deblur and 3D Visualise** will display the Best Focus projection of the data set.
3. Adjust the Min. and the Max. intensities by clicking Options on the **File** menu.
4. Close all views before going on to the next tutorial.

Tutorials for Channels Menu Items

For this tutorial you may use your own data type or you may follow along with the recommended data to use. All multi-channel data, regardless of what microscope it came from or its data type, will be separable into its own channels.

Note: The term Channel is used for delineating data that was collected at different wavelengths for a data set. This menu item will be used for data that is imported as multi-channel data. You may arbitrarily set any of the colour channels to any of the emissive wavelengths collected. The channel description set up below are guidelines for you with respect to which channel will correspond to what wavelength.

Red Channel

To access this feature, click the FILE menu, and select Import RGB Data. The red channel (function) normally represents the longest emissive wavelength collected for the data set.

To select the red channel:

1. From the **FILE** menu, select **Open** or from the Toolbar click on **Open File**.
2. From the Tutorial Data directory select **Multi-channel** folder.
3. Select one of your red channel images and click **Open**.
4. From the **Channels** menu select **Red Channel**. An image set consisting of only the red channel will be generated.

Green Channel

The Green Channel function represents the intermediate length wavelength collected for a 3-channel data set, or the shortest wavelength collected for a two-channel data set.

➡ To select the green channel, open the **Channels** menu and select **Green Channel**. An image set consisting of only the green channel is generated.

Blue Channel

The blue channel (function) represents the shortest wavelength collected for a data set.

➡ To select the blue channel, open the **Channels** menu and select **Blue Channel**. An image set consisting of only the blue channel will be generated.

Note: Close all views before going on to the next tutorial.

All Channels

This function allows you to view the Maximum projection of a Multi-channel colour image.

Multiple probes can be mixed from the **File** menu by clicking **Import Multichannel Tiff….** A Multi-channel image can contain either two or three channels. You can assign any one of the three colours (red, green or blue (R, G, B)) to any one of the channels, when loading a data set. Each pixel in the Multi-channel image will consist of a triple intensity value (R, G, B). This triple value corresponds to the way pixel values are stored. For a two-channel data set you may disable the channel not desired. You may assign any Channel to a sequential data set. The emissive wavelengths and their associated channel colours are suggested for simplicity.

To view the maximum projection of all channels:

1. Under the FILE menu, select **Import Multi-Channel Tiff**, which launches the form that is used to mix multichannel data interface.
2. The Disable Channel selection is **None** by default. This is the setting used for a 3-channel data set. The Channel selection will

be set on Red.

Note: Your Tool's Option in Window's Explorer for "hide file extension for known file types" must be unchecked in order for you to view the *.tif extension.

3. Browse and select one of your green channel images from the multi-channel folder and click **Open**. A dialog appears with the question:
   A sequence of files associated with this selected file is detected. Do you wish to load the entire sequence?
4. Select **Yes**.
5. Select Green from Channel Selection and click Browse.
6. Browse and select one of your blue channel images in the multi-channel folder and click **Open**. A dialog appears with the question:
   A sequence of files associated with this selected file is detected. Do you wish to load the entire sequence?
7. Select **Yes**.
8. Select Blue from Channel Selection and click Browse.
9. Browse and select **Malaria_Blue.0.tif** in the multi-channel folder and click **Open**. A dialog appears with the question:
   A sequence of files associated with this selected file is detected. Do you wish to load the entire sequence?
10. Select Yes.
11. Click **OK** in the **Import Multi-channel TIFF** dialog. A progress indicator will begin showing the importing of the red, green and blue channels. The output from the XY-Max Projection of the three channels is displayed.

Note: For a two-channel data set, select the Blue channel in the Disable Channel box, and navigate to the Red and Green channel locations and load them into the Import Multi-channel TIFF Images that corresponds to the File Locations fields.

Viewing the Channels and Slices of a Multi-channel Data Set

This function allows you to view the channels and slices of a multi-channel data set.

Note: Keep the data set open from the previous section.

To view the channels and slices of a multi-channel data set:

1. Select **Slice Viewer** from the **VISUALIZATION** menu, or click the **Slice Viewer** button. You may navigate through the slices composing the red channel.
2. Close this Slice Viewer projection before going on to step 3.

Note: When a colour is selected from the Channels menu, the view/projection formed depends upon the currently active view/projection.

3. From the View, **Channels** menu, select **Green Channel** or click the Green Channel button to view the green channel.
4. Select **Slice Viewer** from the **Projections** menu, or click the Slice Viewer button. You may navigate through the slices composing the green channel.
5. Select **All Channels** from the **Channels** menu, or click the **All Channels** button to view the Slice Viewer projection of the multi-channel data.
6. Select **Close All** from the **Windows** menu. A dialog appears with the question:
7. **Untitled# is not saved. Do you wish to save it?**
8. Select **No**. This will close all open views.

Tutorials for Settings Menu Items

Image Dimensions

The Image Dimensions are the Width (pixels), Height (pixels) and Depth (slices). The Width, Height and Depth will automatically be set for a data stored as TIFF (*.tif), Leica Deblur and 3D Visualise (*.avz), OcuView (*.ocu), BioRad Pic (.pic), IPLab (*.*), STK (*.stk), Bitmap (*.bmp), or PGM (*.pgm) formats.

The Dimensions will need to be entered by you the first time an 8-bit, 12-bit, 16-bit or 32-bit format data set is loaded.

Spacing (Microns)

The X, Y, and Z Spacing is the size of 1 voxel (in microns) in the X, Y, and Z direction, respectively. The Z spacing is often referred to as the "step size."

- X Spacing is the width of one pixel in micrometers.

Tip: The X Spacing can be calculated by dividing the spatial width of the image frame in micrometers by its width in pixels. X Spacing is entered in micrometers and ordinarily includes a decimal point. An accuracy of 3% or better is required.

- Y Spacing is the height of one pixel in micrometers.

Tip: The Y Spacing can be calculated by dividing the spatial height of the image frame in micrometers by its height in pixels. Y Spacing is entered in micrometers and ordinarily includes a decimal point. An accuracy of 3% or better is required.

- Z Spacing is the sampling distance in micrometers between adjacent optical sections.

Tip: Typically the Z Spacing (step size) between optical sections is selected by the user collecting the data set. The Z Spacing can be determined by dividing the axial depth of the data set in micrometers by the number of optical sections collected.

WARNING: It is critical to have the correct Z Spacing (step size) within a 3% margin of error. This should be measured with a position gauge (for example, Heidenhain or Mitutoyo).

Data Background

For a Darkfield sample the Data Background should be set to Dark, and for a transmitted light brightfield sample the Data Background should be set to Bright.

Deconvolution Settings

Before you are able to deconvolve a data set you must set the Standard Settings and the Expert Settings. The objective of this tutorial is to give you a feel for setting both the Standard and the Expert Settings.

Note: This tutorial contains many explanations and guidelines that are important to the understanding of Standard and Expert settings. It is recommended that you read over the explanations and guidelines to assist you in specifying the Standard and Expert settings.

Standard Settings

The Standard Settings allow you to specify parameters needed for deconvolution. There are three sections:

- Deconvolution methods
- Optics settings
- Deconvolution settings

Inputting these parameters is step one in the deconvolution process.

To input the standard settings:

1. Create an XZ Sum Projection of a suitable data set.
2. Select **Deconvolution Settings** from the **DECONVOLUTION** menu, and click **Standard Settings**. The **Standard Settings** dialog appears.
3. You can now set up the standard settings.

Deconvolution Methods

The Deconvolution Method for Standard Settings allows you to select between Adaptive Blind and Non-Blind.

For Deconvolution Methods to verify the setting, it is set to on Adaptive Blind by default.

Optics Settings

The Optics Settings will prompt you to supply data for the Numerical Aperture of the objective lens and the Refractive Index of the immersion media for the objective lens (oil, glycerol, water or air), which was used during data collection.

Optics Settings also require you to indicate the type of microscope Modality you wish to use. Examples include Fluorescence (Widefield), Brightfield (Transmitted-Light), Laser Scanning Confocal, Spinning-Disk Scanning Confocal, or Multithe-Photon Fluorescence.

On the Image Dimensions tab notice the following:

Value	Pixels	Microns
Width	90	6.3
Height	120	8.4
Depth	50	7.5

➡ To change the Spacings (microns) type the following values:

Field	Value
X Spacing field	0.07
Y Spacing field	0.07
Z Spacing field	0.15

On the **Point Spread Function (PSF) Dimensions** tab The PSF Dimensions fields are disabled. To enable these fields, the **Non-blind method** must be selected, under the **Deconvolution Methods** section. For this tutorial we have selected the Adaptive Blind method.

To set the standard settings by using the Optics method:

1. Select the **Microscope** frame.
2. Ensure that the **Lens NA** value is 1.4.
3. Ensure that the **Refractive Index field** value is Oil (1.515).
4. Ensure that the is selected in the Modality box.
5. Select the **Emissive Wavelength** frame to display the wavelength for each channel.
6. To change the wavelength, select the **channel of interest** radio button, and either type the wavelength (nm) or select the probe type.
7. For the first channel (Channel1) set **Probe** to **FITC** or type 456 for the **wavelength**.
8. For the second channel (Channel 2) set the **Probe** to **DAPI** or type 520 for the **wavelength**.
9. Channel 3 will remain blank when working with a 2-channel data set.

Deconvolution Settings

The Deconvolution Settings section has seven fields:

- Automatic deconvolution settings
- Total iterations
- Save interval
- Output file format
- Deconvolution performance
- Minimum image intensity removal special specimen type
- Use recommended expert settings

To setup the standard settings by using deconvolution methods:

1. Check that the Automatic Deconvolution Settings are disabled.
2. In the **Total Iterations** field type a value of 100.
3. In the **Save Interval** field type a value of 50.
4. This will perform 100 iterations of deblurring, saving the 50th and 100th iteration.
5. In the **Output File Format** verify that the setting is on TIFF (16-bit). This is the same format as for the Input File.
6. Click the **Best Medium Fast** check box.
7. The Deconvolution Performance settings refer to the "Stability, Resolution, and Speed" of the deconvolution.
8. Verify that **Noise Level** is set to No.
9. Verify that the **Special Specimen Type** is blank.
10. Verify that the **Use Recommended Expert Settings** is enabled.
11. Click **OK**.

Total Iterations

The Total Iteration field under Deconvolution Settings allows you to choose the number of iterations performed during a deconvolution. The higher this number the more times the data will be processed. Lower iterative numbers will execute in less time whereas the higher iterative numbers will take more time and generally provide improved resolution. Beyond a certain number, depending upon the imaging modality (widefield or confocal) the deconvolution may provide unstable results.

When you wish to use either Widefield Data or Confocal Data, the following guidelines will help you choose the appropriate number of **Total Iterations** and the deconvolution performance setting.

For Widefield data you are recommended to use **Best High Moderate** for the **Deconvolution Performance** setting and start with a value of 80 for the number of **Total Iterations**. Consider the following criteria when selecting a number of Total Iterations:

Values	Results
1 - 80	Minimum: use if speed is the most important consideration.
81 - 100	Recommended as initial range of values.
101 - 350	Maximum recommended under normal conditions: use if resolution is the most important consideration.
351 - 500	The higher iteration settings may provide superior resolution, but require a very low noise level.

If you choose **Faster Processing** by ticking **Performance** you may start with a value of 40 for the number **Total Iterations**. Consider the following criteria when selecting a number of Total Iterations:

Values	Results
1 - 40	Minimum: use if speed is the most important consideration.
41- 70	Recommended: use for optimal trade off between speed and robustness.
71 - 100	Maximum recommended under normal conditions: use if resolution is the most important consideration.

Save Interval

The Save Interval field allows you to select the number of iterations that will occur between storage of deconvolution results on the disk. The possible values are integer from 1 to the number of Total Iterations. For example, if the number of Total Iterations is 20 and the Save Interval is 5, the deconvolution will be saved at 5, 10, 15 and 20 iterations. The deconvolution application automatically checks for available disk space before beginning deconvolution.

Output File Format

The **Output Settings File Format** field allows you to select the format in which deconvolution application will save the deconvolution results. The formats available for deconvolution are:

- TIFF (16 bit)
- TIFF sequence (16 bit)
- SEQ
- STK

Use the **Leica Deblur and 3D Visualise** File (*.deb) to retain the most accuracy and dynamic range, especially if quantitative accuracy is important. For visual output, typical monitors only support 256 greyscale levels, so the 8-bit selection may be acceptable if quantitation and dynamic range are not important. The TIFF format allows the deconvolution results to be easily imported into other software. The TIFF format uses no compression and is an 8-bit format. The **Leica Deblur and 3D Visualise** File (*.deb) output will require four times the disk space of an 8-bit or TIFF output.

Deconvolution Performance

The Deconvolution Performance field allows you to select from two Deconvolution Performance settings. These settings affect the performance with regard to the stability, resolution, and the speed of the deconvolution. You can choose how quickly the results are returned versus the resolution desired.

The **Unchecked** setting, which refer to stability, resolution and speed respectively, allows the deconvolution to have the highest stability and resolution. It is generally used when working with relatively thick samples or noisy data sets. The **Checked** setting provides higher speed with a small trade-off in resolution.

Minimum Image Intensity Removal

Minimum Image Intensity Removal automatically calculates and removes the erroneous background intensity level in the data. The

most common cause of this erroneous background intensity is the electronic dark current (background electrical signal level) of the photodetector or CCD camera. Other causes are the bias voltage of amplifiers in the camera, back-scattered light that penetrates the emission filter and non-specific dye that may leak into the embedding medium, among other causes. You can enable Minimum Image Intensity Removal by setting the tick box that is found on the **Expert Settings** form that is a sub menu of Deconvolution Settings.

Use Recommended Expert Settings

You are recommended to use the **Use Recommended Expert Settings** option.

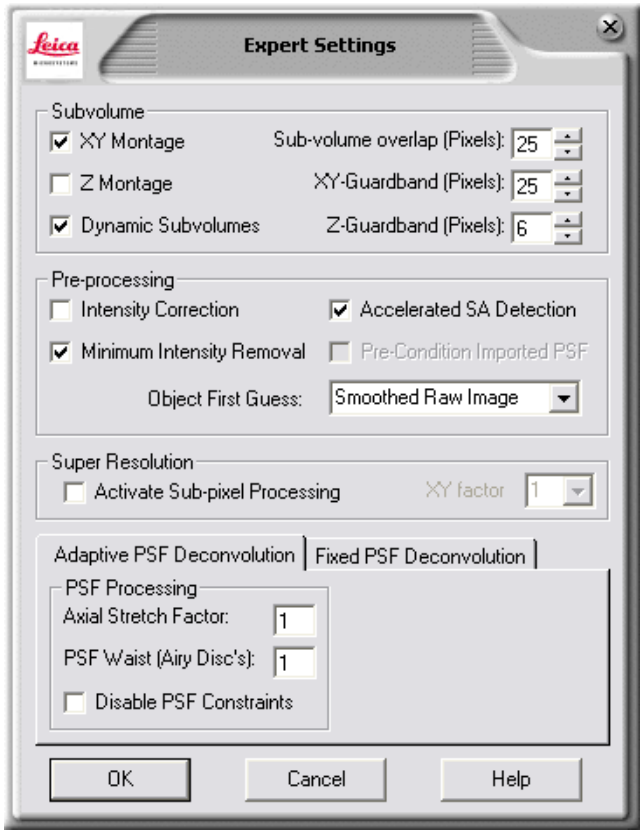
WARNING: As a general rule, DO NOT MODIFY THE EXPERT SETTINGS until you have attained expertise in using the deconvolution application.

Do not adjust the Expert settings from their original default settings except in rare cases. Do not change them casually. All adjustments should be tested on a small sub-field of the data set (for example, 64 x 64 x 32).

➡ If you disable the **Use Recommended Expert Settings** (uncheck the check box), the **Go to Expert Settings** button becomes active.

Expert Settings

➡ With a suitable data set loaded, from the **Settings** menu select **Deconvolution Settings** and click on **Expert Settings**. The **Expert Settings** dialog appears with two sections: the Object section and the PSF section.



Object Section

This section of Expert Settings affects the handling of the object data. The default values are:

- Z Montage is deselected (disabled)
- XY Montage is selected (enabled).
- Dynamic Subvolumes is selected (enabled).

Leave these fields at their default values.

Z Montage

The Z Montage setting allows the deconvolution application to break the data set into sections along the optical axis and to deconvolve these subsections separately. The valid settings are:

- On (checked)
- Off (unchecked)

The default for this option is “Off”. It should only be turned “On” for image stacks with a large “Depth” setting (for example, >100 slices). This option reduces the amount of RAM required by the deconvolution process. It may also be useful in rare cases where the sample thickness is so large that the PSF changes dramatically along Z. In such cases, Z Montage allows the blind deconvolution to find different PSF solutions for different depths.

XY Montage

The XY Montage option allows the deconvolution application to break the data into sub-volumes along the XY dimensions. The valid settings are:

- On (checked)
- Off (unchecked)

The default for this setting is “On”. This option reduces the amount of RAM required by the deconvolution application. It should be turned “Off” only if the deconvolution application is producing rigid, box-like artefacts in your data.

Dynamic Subvolumes

The Dynamic Subvolumes selection allows the deconvolution application to subdivide the data set into the largest size the processing computer’s RAM can handle. The advantage of larger subdivisions of data being processed is the increase in processing speed and therefore a decrease in the amount of time it takes to deblur a data set.

You should verify that the following fields are set to their default values:

- The Subvolume overlap (Pixels) is 10.
- The Guardband (Pixels) is 10.
- The Z-Guardband (Pixels) is 6.

Subvolume overlap

The Subvolume overlap setting (Pixels) determines the number of pixels that the montaged subvolumes will overlap. The possible values are integers from 0 to N/2, where N is the width or height of the XY field in pixels whichever is smaller.

An overlap of 10 or 25 pixels usually works best. If the result of the deconvolution contains artefacts having rigid lines, edges or an obvious grid structure start with a value of 10. If doing so reduces the problem, but does not eliminate it, then increase this number again. Overlapping regions are deconvolved twice, so making this number too large (for example, 100) will increase the deconvolution time.

Guardband (Pixels)

The Guardband size defines the width of a border surrounding each subvolume. This border is the region where the subvolumes are processed to seam them together. This guardband prevents artefacts at the seams. The possible values are integers from 0 to N/2, where N is the width or height of the XY field in pixels whichever is smaller.

Generally, the larger the guardband, the fewer the artefacts and the better the image quality. However, deconvolution time increases with guardband size, so a default value is set which minimises deconvolution time and eliminates artefacts in most cases. The default value is 10. This number may be increased if seaming artefacts appear. If so, first increase the number to 15, then 20, and then 25 until the seaming artefact is gone.

Z-Guardband (Pixels)

The Z-Guardband specifies the number of slices that will be added at the top and bottom of the subvolume. This Guardband prevents artefacts at the seams of these subvolumes. The possible values are integers from 0 to N/2, where N is the depth of the XZ or YZ field.

The Z-Guardband should never be larger than the subvolume overlap region. A value of 6 is adequate for most image stacks.

Leave the default setting of Low for the Noise Smoothing setting.

Noise Smoothing

Noise Smoothing allows you to indicate the amount of noise you see in the data. The **Noise Level** control on the **Standard Settings** form allows you to adjust this for different data. A setting of Low indicates that noise can barely be seen in the data. A setting of Medium indicates that the noise can easily be seen in the data, and a setting of High indicates there is a significant amount of noise.

- Widefield data generally contains low levels of noise, therefore the default setting is **Low**.
- Confocal data generally contains medium levels of noise, therefore the default setting is **Medium**.
- Use **Other** to manually input the Noise Smoothing Factor to indicate that the amount of noise falls in between the settings of **Low**, **Medium**, or **High**.

PSF section

In the PSF section there are two tabs:

- Adaptive Blind
- Non-Blind

Non-Blind tab

The Non-Blind tab allows you to select the PSF input from two choices: Synthetic PSF or Measured PSF.

Adaptive Blind tab

In the First Guess frame, verify that:

- Stretched Theoretical is enabled.
- Axial Stretch Factor is set to 1.

The First Guess frame has two options:

- Flat Sheet that selects the initial estimate of the object used to initiate a blind deconvolution process. This option uses for first

guess, a volume filled with a constant value equal to the average of the entire image stack. For very noisy data, this is an appropriate starting point for the deconvolution.

- Stretched Theoretical that is a theoretical (calculated) PSF lengthened along the Z-axis to more accurately initiate the first guess for a non-widefield data set.

➡ Verify that the Disable PSF Constraints is disabled. This removes the limitations placed on PSF.

➡ Verify that the PSF Waist is 1. This is the size of the narrowest part of the PSF, usually measured in Airy Disc diameters. The default settings are 1 for Widefield and 3 for Confocal.

➡ Click **OK** to close the **Expert Settings** dialog.

Operation Settings

The Operation Settings in the main File menu allow you to specify information regarding the File directory and the Rendering Range for various projections of a data set.

Files

You can choose the location of the temporary files or the startup directory by either typing in the new location or browsing to the new location. The startup directory is the location in which the software application will look for image sets.

Note: You are advised to place the temporary files directory on a drive with at least 1-gigabyte of free space.

Rendering

The Rendering tab allows you to set the range of values a pixel may have depending on the projection chosen or the threshold values for a projection. The projection rendering ranges can be set for Max.,

Min., and Sum Projections. The threshold values can be set for Voxel Gradient, Best Focus, and Alpha Blending projections.

Note: You are advised to leave the Rendering settings at their default values. You may want to change the Rendering settings in the future for noisy or photo-bleached data sets.

The default Rendering Range values are:

Values	Results
0% to 100%	The Max. Projection Rendering Range
0% to 100%	The Min. Projection Rendering Range
0% to 100%	Sum Projection Rendering Range
20% to 100%	The default Rendering Range of Voxel Gradient, Best Focus and Alpha Blending

The default Alpha Value is 0.5.

Load Settings

This feature allows you to load settings previously saved using the Save Current Settings As feature. Load Settings include all fields found in the Standard and Expert Settings.

Save Current Settings As

This feature allows you to save the Standard and Expert Settings as text files for later use with another data set. The file is saved with an (*.set) extension.

This section of the tutorial assumes you have the FitcDapi_crop.tif data set open and that the Standard and Expert parameters have been set up as described in the proceeding sections on Standard Settings and Expert Settings.

To save the current settings:

1. With your data set as the active view, from the **Settings** menu select **Save Current Settings As**.

2. In the **File name** field type the name you would like for these settings. For example, Test_parameters.set.
3. Click **Save**. You have now set and saved the Standard and Expert Settings for deconvolution.
4. Close all views before going on to the next tutorial.

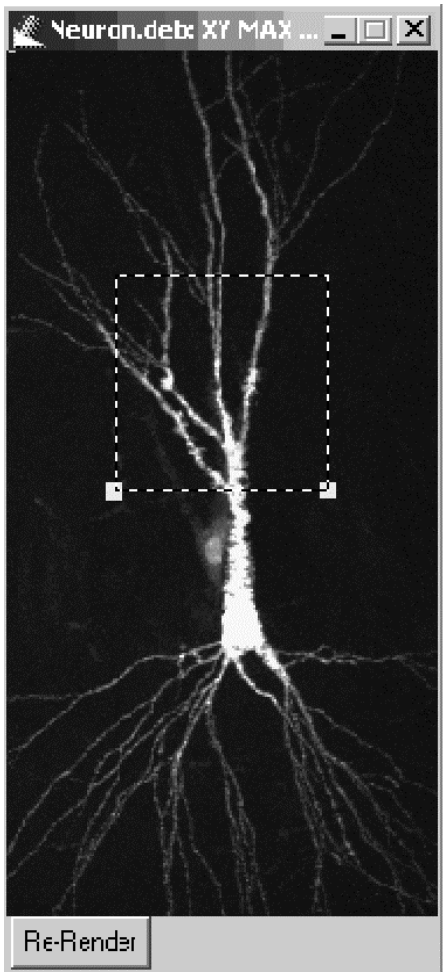
Tutorials for Operations Menu Items

Crop the Stack

This function takes a selected region of an image (see Select Region), and creates a new document based on the pixels that fall inside that selected region.

To crop the stack:

1. Start in upper left corner of the view to select a region.



2. Press the left mouse button down and simultaneously drag the mouse to form a square similar to the one demonstrated in the

figure above. Once the square is formed, release the left mouse button. The size of the above cropped area is approximately 100 by 100 pixels.

Note: The cropped region's dimensions are concurrently displayed in the lower left corner on the main window status bar.

3. Select **Crop** from the **Operations** menu, and click on the **Crop the Stack** option or from the Toolbar click on the **Crop** button. The image will be cropped and displayed in the viewing window.
4. Select **Save As** from the **File** menu, type a file name to save the cropped image.
5. Click **Save**.

Slice by Slice Cropping

To crop using the slice by slice method:

1. Open a suitable data set in a slice viewer, and from the **Operations** menu choose **Select Region** and select the **Rectangle** as the cropping tool.
2. Click within the **Slice Viewer** projection and place your cursor in the upper left corner of the area of interest. Click and hold down the left mouse button while dragging your cursor to the right and downwards. A dotted-line rectangle will be created.
3. Select **Crop** from the **Operations** menu, and choose Slice by Slice Cropping. The Cropping Mode Selection dialog appears.
4. Press the **Apply Region** button. An unchecked check box appears to the left of the number 1 in the list box. This represents the first region of interest selected.
5. Click on the check box next to the number 1 to select it. A highlighted overlay appears in the selected region.

Note: You may now choose the Crop function or the Remove Region function.

Crop function	Crops out the previously selected region of interest and will display the cropped data as a new data set.

Remove Region	Removes the region of interest from the data set, and will display the removed region as black within the data set. This function is useful in removing artefacts from data sets.

You may select from one of three following options:

- Apply to current slice only allows you to select a region of interest on the current slice.
- Apply to all slices takes the selected region of interest on the current slice and applies it to all of the slices in the data set. A new volume is created from the cropped area of interest.
- Apply to given slice range takes the selected region of interest and allows you to apply it to a specified range of slices. The newly created and cropped volume will only contain the slices you specified.

To crop a region of interest:

1. For this tutorial select **Apply to given slice range** option and type18 for the **From slice** and type 50 for the **To slice**.
2. Press the **Crop** button. A new Untitled# data set of the cropped region appears in a Slice Viewer projection.
3. Repeat the steps above and choose Remove Region (see step 7 to remove a region of interest.

Tip: If you desire to Crop out a different shape on each slice (or only on a few slices) you should select the Apply to current slice only choice. The **Apply Region** button must be pressed to have the current selected region take part in the Crop or Remove Region.

When a region is selected for cropping and the check box is checked, the first highlight will be light blue. Select another slice using the scroll bar at the bottom of the Slice Viewer and repeat the steps of selecting a region, pressing the **Apply Region** button and checking the box left to the number in the list box.

Note: The Clear Selected Regions button will remove the crop overlay from the regions that are currently selected.

4. When you have chosen all of the desired cropping regions, press the **Crop** button. A cropped volume will be displayed with

only the foreground image data from the highlighted regions; all else will be set to background.

Note: Do not close Neuron.deb. Continue on to the next section.

Extend Slices

An image can be extended (have additional optical slices added to it) along its Z-axis.

This function allows you to generate additional image slices based on linear interpolation, and appends them to the top and bottom of an image. It is not the same as adding blank slices to the top and bottom of a stack as mentioned below.

An extended data set will have false slices attached to its top and bottom. These false slices serve the purpose of a buffer to keep the guardband region from overlying meaningful features, thereby keeping the meaningful data from being obscured in the deconvolution process.

This tutorial continues with the Neuron.deb data set from the Crop tutorial above.

To crop extended slices:

1. Select **Extend Slices** from the **Operations** menu, or click on the Extend Slices button on the Toolbar. The **Extend Image** dialog appears.

Note: You have the option of extending the data by using false slices or by using the Zero Pad option which when selected gives you the ability to add blank slices devoid of any information.

2. In the **Extend Depth** box type the value 4 for the Top field value.
3. In the **Extend Depth** box type the value 4 for the Bottom field value.
4. The new Depth value will be displayed in the New Dimension frame.
5. Leave the **Zero Pad** box unchecked.

6. Click **OK**. The extended data set will be displayed. Click on the XZ view button, and notice the difference between the real data slices and the false slices just generated.
7. Close all views before going on to the next tutorial.

Optical Density Correction

This feature corrects fluctuations in the image intensity values across the depth of an image. This only works on images with depth > 1. The image intensity value often fluctuates erroneously because of random flicker from the camera shutter or the lamp instabilities. Flicker occurs with nearly all widefield microscope systems. It is due to several causes. Most Cooled CCD cameras have a randomness in their shutter's speed. This shutter speed fluctuation causes variations of the order of several milliseconds (typically) from one exposure to the next. The effect can be seen best in a side-view projection of a data set.

Tutorials for Movie Menu Items

Leica Deblur and 3D Visualise allows you to create a movie sequence. To create a movie, a series of frames are generated and then displayed in a contiguous manner. A movie sequence consists of a series of rotations through a specified range of images and about a specified axis. You may also set contrast settings, the type of the projection, the number of steps, the Start and End Angles, and the Anchor axis (axis of rotation). The movie generated can be played later in **Leica Deblur and 3D Visualise** or exported as an AVI file.

Movie Maker

The Movie menu of the 3D Viewer provides the tools needed to generate a movie. You have two options to generate a movie:

- Use the Quick Movies option under the Movie menu, which allows you to generate a movie "quickly" by spinning the data about the X or Y axis by predetermined angles (+/- 30, 45, 60, 90 or 180 degrees).
- Generate a movie by setting your own Start Point, Mid Point, End Point, and Step Angles.

To create a movie:

1. Select **Movie Maker** from the **Movie** menu. The **3D Previewer** dialog appears.

Note: Images in the 3D Previewer will open in the XY view only

Note: This tutorial continues by using the Quick Movies option.

2. Within the **3D Previewer** dialog click on the **Movie** menu and select **Quick Movies**. Click on **Rotate Y Axis** and select the **+/- 60 Degree** option. This will generate a movie that rotates about the Y-axis by +/- 60 degrees in the Rock mode. To **stop** the Movie at any time, click on the **Movie** menu again and select **Stop**

Movie. To play the movie again you may click on **Play Movie** from the **Movie** menu.

3. You may play the movie in Loop mode by selecting **Loop mode** from the **Movie** menu and then choosing **Play Movie**. If you wish at any time to play the movie backwards you may do so by selecting **Opposite Path** from the **Movie** menu and then clicking on **Play Movie**. To restore your original image select **Original View** from the **Quick Movies** option from the **Movie** menu of the **3D Previewer** dialog.
4. Restore your original view by selecting **Original View** from the **Quick Movies** options.
5. Close all views before continuing on to the next section.

To generate a movie by selecting your own Start Point, End Point, and Step Angle:

1. Choose your **Start Point** by either starting with the original view or by rotating your data with your mouse to a position that you want the movie to start from.
2. Select **Start Point** from the **Movie** menu.
3. Use your mouse to rotate the view to a position that you want as the end point for the movie. Select **End Point** from the menu.
4. Select a **Step Angle** from the available options under the **Step Angle** option from the **Movie** menu.
5. Click on **Play Movie**. The movie will play in the direction in which you selected your Start Point and then the End Point.
6. Again, you may play the movie in **Loop mode**, **Rock mode**, and in the **Opposite** direction.
7. Close all views before continuing on to the next section.

Note: You may also select a Mid-Point when generating a movie. To do so follow the same steps as explained above for setting the Start Point and End Point.

The last option under the Movie menu is **Create a Movie**. This feature opens an untitled movie file, which may be saved. It provides all the options to Play the movie, Stop the movie, Rock mode Play, and Loop mode Play. Before you are able to access the created movie file you must close the 3D Viewer dialog.

Open a Movie

To open (or play) a movie:

1. Select ***.avm** from the **Movie** menu. Set the Files of type to *.avm.
2. Select **Colrpollenrs.avm** from the **Movies** directory, and click **Open**. The first frame of the movie is displayed.

Note: The *.avm file extension indicates a **Leica Deblur and 3D Visualise** movie header file. This header file contains the necessary information to playback the movie. By selecting this file the entire movie will be loaded.

3. Press the **Play** button to play the movie. The movie plays once through then automatically stops.

4. To stop a movie that is currently playing press the **Stop** button.

5. Press the **Loop Mode** button to put the movie playback into Looping Play. Press the **Play** button and the movie will continuously play in a Looping motion until the **Stop** button is pressed.

6. Press the **Rock Mode** button to put the movie playback into Rocking Play. Press the **Play** button and the movie will continuously play in a Rocking motion until the **Stop** button is pressed.

Note: Do not close the Colrpollenrs.avm movie. Continue on to the next section.

Concatenating Movies

This tutorial continues with the movie from above in the Open Movie section.

To concatenate movies:

1. Select **Concatenate Movies** from the **Movies** menu.
2. The **Concatenate Movies** dialog appears. Click the **Add a Movie** button and navigate to the directory where the movie files are stored.
3. Select the first movie to be added in the concatenation and click the **Open** button. The movie will be placed first in the Movie List.
4. Click the **Add a Movie** button again and select the another *.avm to be opened. Once the Open button is clicked, **Leica Deblur and 3D Visualise** will add the *.avm to the Movie List in the second place.

Note: If you make a mistake in selecting a movie, the movie may be easily removed by selecting the movie and clicking the **Remove** button. **Leica Deblur and 3D Visualise** will remove the highlighted movie from the Movie List. For now, leave both movies in the Movie List.

5. Click the **OK** button and **Leica Deblur and 3D Visualise** generates a new movie that comprises both movie files. This concatenated movie can then be saved and concatenated again with another movie if desired.
6. Click the **Loop Mode** button on the Movie player bar and then click the **Play** button. **Leica Deblur and 3D Visualise** displays the frames of the movies in a continuous Loop pattern.
7. Click **Stop**.
8. Close all movies before continuing on to the next section.

Tutorials for Start Deconvolution

Start 3D Blind Deconvolution

Blind Deconvolution is considered to be the most accurate algorithm available with **Leica Deblur and 3D Visualise**. It does not require the calibration and measurement of the Point Spread Function (PSF).

3D Blind Deconvolution is an iterative and constrained algorithm. It is iterative in the sense that it repeats the same computational operations many times while converging to the enhanced image solution. It is constrained in the sense that it only accepts deconvolved images that have the correct mathematical properties of non-negativities, (that is, it does not allow the tracer concentration to have negative values) and smoothness (suppresses snowy-like noise due to low-light levels). 3D Blind Deconvolution is a method of deconvolution that adapts itself to the real PSF of the microscope system (which can be significantly different from the theoretical PSF and from the previously measured PSFs) due to specimen and instrument variations.

The **Leica Deblur and 3D Visualise** Blind Deconvolution system adapts to PSF changes within a specimen itself. Thus, the deconvolved results are superior to those methods which utilise theoretical or previously measured PSFs, which do not require the point spread function (PSF) of the system to be *explicitly known prior* to the deconvolution.

Using 3D blind deconvolution:

1. With an appropriate data set loaded, select Launch 3D Deconvolution from the Launch menu. The Launch 3D Deconvolution dialog appears.

Tip: Before starting a deconvolution, it is good practice to verify the Deconvolution Settings.

2. On the **Image Dimensions** frame verify the following:

Dimensions	Pixels	Microns
Width	90	6.3
Height	120	8.4
Depth	50	7.5

3. Verify the Spacings values are:

Dimensions	Microns
X	0.07
Y	0.07
Z	0.15

4. In the **PSF Dimensions** frame ensure that the PSF Dimensions fields are disabled. To enable these fields, select the Non-blind method from the **Deconvolution Methods** section.

Note: For this tutorial we have selected the Adaptive Blind method.

5. On the Microscope frame:

Field	Selection
Numerical Aperture field	1.4
Refractive Index	Oil (1.515)
Modality dialog	Fluorescence

6. In the **Emissive Wavelength** frame, check that the wavelength for each channel is displayed.
7. Check that the **channel1 (**first channel) is set with the correct Probe type and wavelength.
8. Check that the **channel 2** (second channel) is set with the correct Probe type and wavelength.
9. Check that the **Automatic Deconvolution Settings** is disabled.
10. Type a value of 100 into the **Total Iterations** field.
11. Type a value of 50 into the **Save Interval** field enter. This performs 100 iterations of deblurring, saving the 50th and 100th iteration.

12. In the **Output File Format** check that the setting is on TIFF (16-bit). This is the same format as the Input File.
13. Tick Performance checkbox for faster processing. Set the Noise Level.
14. Check that the **Minimum Image Intensity Removal** is set to No.
15. Check that the **Special Specimen Type** is blank.
16. Check that the **Use Recommended Expert Settings** is enabled.
17. Click **OK**.
18. The 3D Blind Deconvolution starts. The results are displayed in the main viewing window.
19. Close all views before continuing on to the next section.

2D Deconvolution

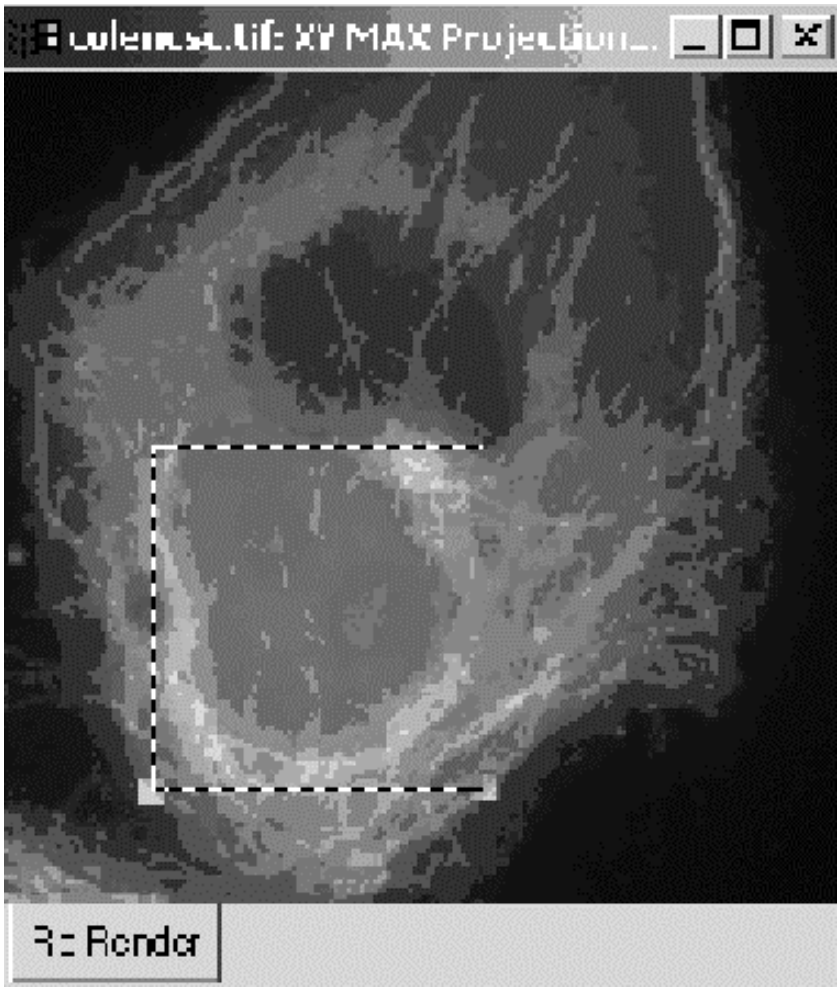
Leica's Deblur and 3D Visualise deconvolution allows you to apply the Blind (Adaptive) Deconvolution to a single two-dimensional image. The 2D Deconvolution algorithm is also capable of improving the resolution of an image for the restoration of features at a sub-pixel resolution level. You may process multi-frame (time series) image sets, individual colour channels or intensity images. The 2D algorithm is able to suppress noise while retaining quantitative accuracy (total number of photons) in the image, thus allowing you the ability to make valid quantitative measurements. The new high-speed deconvolution algorithm in the 2D Deconvolution decreases processing time by a factor of five or more.

Setting Up a 2D Deconvolution

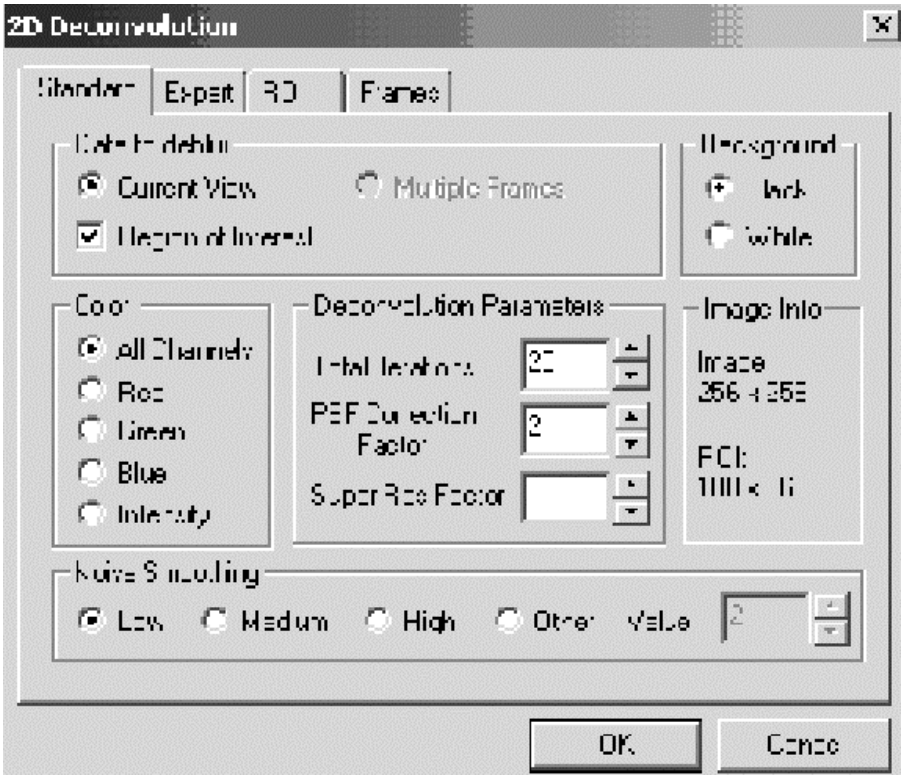
Select a Region of Interest

To select a region of interest:

1. Click and hold down the left mouse button, while dragging out a Region of Interest (ROI) similar to the one shown below.



2. Select **2D Blind Deconvolution** from the **Deconvolution** menu. The **2D Deconvolution** dialog appears, with the **Standard tab** as the active tab.



3. Set the parameters to the following values:

Selection	**Setting**
Data to Deblur	Region of Interest box already checked
Colour selection	All Channels
Deconvolution Parameters	Increase the Total Iterations number to 30
PSF Correction Factor	Unchanged
Super Res Factor	Unchanged
Image Info dialog settings	Unchanged
Noise Smoothing	Low
Expert tab	Default
ROI tab	Default
Frames tab	Default

Note: The image size defined in the Image Info dialog settings are measured in pixels, that is 256 x 256.

Note: The Noise Smoothing setting should remain as Low, as this image

does not contain a great amount of background noise.

4. Click **OK** to begin the deconvolution process.

Note: To interrupt the deconvolution, press the ESC key and wait for the current iteration to finish.

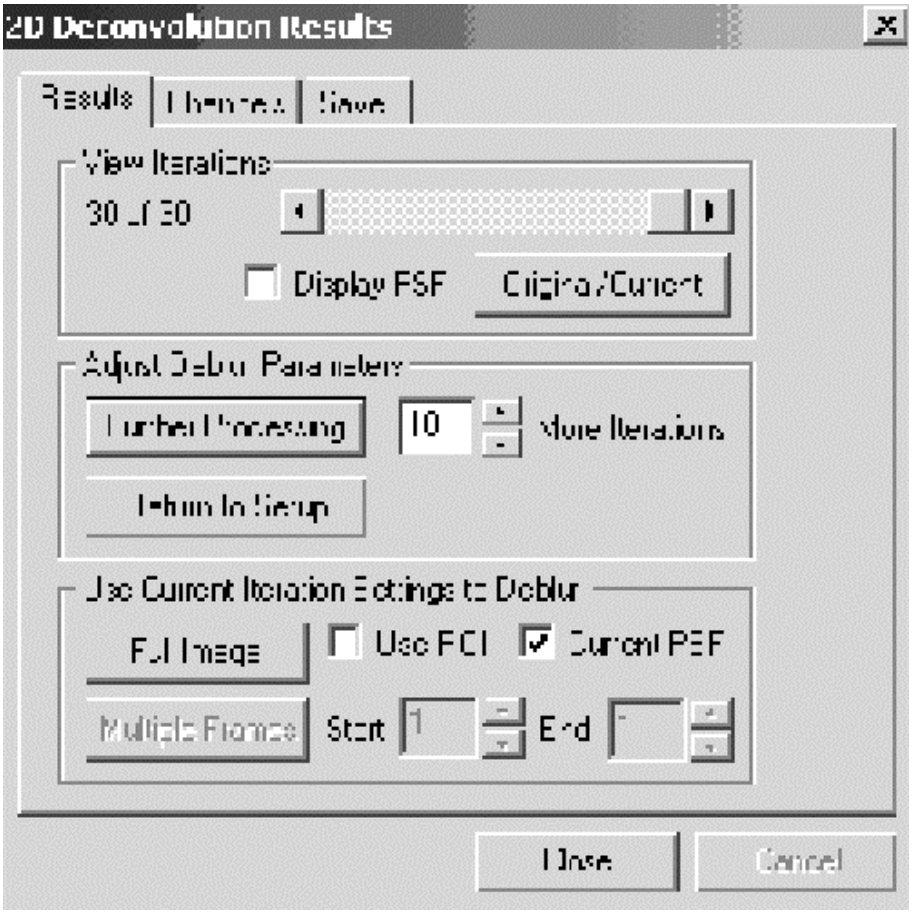
5. When the 2D Blind Deconvolution is complete the **2D Deconvolution Results** dialog appears with three tabs: Results, Channels, and Save.

2D Deconvolution Results

In the Results tab, you are able to view the result of the deblurring process at each iteration by using the scroll bar in the View Iterations field. You have the option to display the PSF during the viewing process by checking the check box next to Display PSF. You also have the option to alternate between the current iteration number result and the original image by clicking on the Original/Current button.

Within the **Adjust Deblur Parameters** dialog you may choose to do further iterations by clicking on the **Further Processing** button or you may return to Setup to rerun the 2D Deconvolution with different parameters.

Within the **Use Current Iteration Settings to Deblur** dialog you may choose to process the Full image or Multiple Frames of the Full Image or ROI (Region of Interest). You may click the **Use ROI** dialog to enable it and then click the **Full Image** button to process that region of interest throughout the entire data set.



Time Lapse Images

You also have the option of selecting a region of interest and applying the region of interest to only part of a time lapse data set.

To apply an ROI to only part of a time lapse data set:

1. Select **2D Deconvolution** from the Launch menu. The **2D Deconvolution** dialog appears with the **Standard tab** displayed.
2. Select the **Multiple Frames** option from the **Data to Deblur** frame. To set the range of images you would like to process, select the Frames tab and in the Multiple Frames frame select Frame range.
3. Type the desired starting Frame number (image number) in the Start Slice dialog, and type the desired ending Frame number (image number) in the End Slice dialog.
4. When you click **OK,** the **Save Multi-Frame Deblur** dialog appears. Use the default name for the resulting data set or type

a name for your resulting data.

5. Click on the **Save** button. A 2D Deconvolution will run on your data set, and upon completion you will be prompted to save the newly created file. After you have clicked on the **Save** button another dialog appears asking you: *Do you wish to export as multiple files, each of a single slice?*
6. If you select **Yes**, **Leica Deblur and 3D Visualise** shows the naming extension it will append to the data set. The new data set will be saved under a name indicating the Start and End slices along with the number of iterations in its title.

Inverse Filter

Use the Inverse Filter feature with Widefield Fluorescence and Transmitted Light brightfield data sets only.

The Inverse Filter is a one step non-iterative deconvolution method based on inverse-filtering theory. It utilises optimal linear filtering.

Inverse Filter is one of the simple deconvolution methods offered by **Leica Deblur and 3D Visualise**. The feature is useful for obtaining quick results, but is not as accurate as Blind Deconvolution. Inverse Filtering is typically more robust than the Nearest Neighbours or No Neighbours deconvolution methods. The execution speed of the Inverse Filter is between that of Nearest Neighbours and Blind Deconvolution.

The Inverse Filter should be used in cases where speed is important. A typical processing time is under two minutes with a 256 X. 256 X. 32 data set (Pentium III, 450 MHz).

To use inverse filter deconvolution:

1. Select **Deconvolution Settings** from the **Settings** menu and choose **Standard Settings**. Double click within the image to display the **Standard Settings** dialog.
2. Check the following dimensions for the **Image Dimensions tab**:

Dimensions	**Pixels**	**Microns**
Width	90	32.724

Height	80	28.56
Depth	110	44

3. Change the **Spacings** (Microns) by typing the new values:

Dimensions	**Microns**
X	0.3636
Y	0.357
Z	0.4

4. In the **Microscope frame** check the values are:

Dimensions	**Selection**
Numerical Aperture	0.7
Refractive Index	Oil (1.515).
Modality box	Fluorescence
Emissive Wavelength frame	Probe left blank
Channel 1 wavelength	540nm
Channel 2	Blank
Channel 3	Blank

5. Click **OK** on the Standard Settings tab.
6. Select **Inverse Filter** from the **Launch** menu. The **Inverse Filter Parameters** dialog appears.
7. The **Image Dimensions frame** contains the data set's Width, Height, Depth and Spacings. Check that they are the same as listed above. The Phase Content Expected should be used if the specimen is a Transmitted Light Brightfield data set, and exhibits significant phase characteristics, for example, areas of the specimen appear brighter than the background. For this data leave the Phase Content Expected dialog unchecked.

8. Click **Start**. **Leica Deblur and 3D Visualise** executes the Inverse Filter deconvolution. The status bar indicates the progression of the Inverse Filter.
9. When the Inverse Filter method has finished, a new **XY-Max Projection** view appears. This is the result of the Inverse Filter deconvolution. Do not close the Inverse Filter result Untitled data set. Compare it to the results of the Nearest Neighbours deconvolution from the next section.

Nearest Neighbours/No Neighbours

Use Nearest Neighbours/No Neighbours with Widefield Fluorescence and Transmitted Light brightfield data sets only.

The Nearest Neighbours algorithm is the fastest algorithm available. It works by deconvolving one image slice at a time. As a trade-off in order to achieve this speed, it is less accurate than either the Blind Deconvolution or the Inverse Filter. It should be used in cases where speed is most important. Typical processing times are less than “1” second for a 256 x 256 single image slice and less than “1” minute for a 256 x 256 x 32 3D data set (Pentium III, 450 MHz).

Leica Deblur and 3D Visualise contains two methods for running the Nearest Neighbours deconvolution on a data set:

- Processing Stack that runs the specified deconvolution on the entire image stack.
- Processing Current Slice that is run while viewing one slice of an XY-Slice Viewer of the image stack.

Note: Do not close the Pollen.deb data set. Continue on to the section.

Processing Current Slice

To process the current slice:

1. Select **Slice Viewer** from the **Projections** menu. The slice currently displayed will be the slice processed.
2. Select **Nearest Neighbors** from the **Launch** menu and click on **Processing Current Slice**. The **Nearest/No Neighbor Slice Operation** dialog appears. The Haze Removal Factor, the Z Kernel Width, and the No Neighbours selections will be set at their default values.
3. Click the **Apply** button. The Nearest Neighbours deconvolution is applied to the current slice.
4. Click the **Restore** button to return to the original view of the slice.
5. To use the No Neighbours algorithm instead of the Nearest Neighbours algorithm check the **No Neighbors** box on the **Nearest/No Neighbors Slice Operation** dialog.
6. Adjust the results by trying different values for the **Haze Removal Factor** and the **Z Kernel Width**, also try the No Neighbours deconvolution by clicking in the **flag** box to activate and deactivate it.
7. Set the **Haze Removal Factor** to 0.97, the **Z Kernel Width** to 3, and leave the **No Neighbors** box unchecked. These values produce satisfactory results for the Nearest Neighbours or No Neighbours algorithm.
8. Click the **Restore** button to restore the optical section to its original view.
9. Click the **Close** button to exit from the **Nearest/No Neighbours Slice Operation** dialog. The algorithm chosen (Nearest Neighbours), and the values selected for the Haze Removal Factor and the Z Kernel Width are now saved for this specific data set.

Note: Do not close the Pollen.deb data set. Continue on to the section.

Processing Stack

To process a stack:

1. Select **Nearest Neighbours** from the Launch menu and select **Processing Stack**.
2. The **Nearest/No Neighbors Parameters** dialog appears. The settings for the **Haze Removal Factor** (0.97), the **Z Kernel Width** (3), and the **Nearest Neighbours algorithm** were determined above in the Processing Current Slice section. The Phase Content Expected should only be checked if the specimen is a Transmitted Light Brightfield data set, and exhibits significant phase characteristics, for example areas of the specimen appear brighter than the background. For this data set leave the **Phase Content Expected** box unchecked.
3. Click **Start**. The deconvolution will be performed on each slice of the entire stack. A status bar will indicate the progression.
4. Compare the results of the Nearest or No Neighbours deconvolution with the original data and note how the deconvolution removes haze and sharpens features. Examine the Maximum projections and the Slice Viewers of the original Pollen.deb data set, the Inverse Filter, and the Nearest Neighbours results in both the XY and XZ views.
5. Close all data sets before continuing on to the next section.

Axial Blind Deconvolution

Note: Use only with Confocal data.

Axial blind deconvolution (also known as 1D deconvolution) is a form of blind deconvolution especially customised for speed. It is designed to be the fastest method for deblurring a confocal data set, and is used for applications where improving the resolving power is desired only along the optical axis. This is a typical scenario for confocal data collection where empty lateral magnification is often avoided (the X and Y dimensions show no distortion or haze). Typical processing

times are in a range from two to five minutes with a 256 x 256 x 32 data set (Pentium III, 450 MHz).

To deblur a confocal data set:

1. Select **Axial Blind Deconvolution** from the **Launch** menu. **Leica Deblur and 3D Visualise** executes a one-dimensional deconvolution of the data set. A status dialog appears indicating the progression.
2. The Untitled1 deconvolution results appear on the screen upon completion.
3. Generate an XZ View by clicking on the **XZ view** button on the Toolbar. Untitled1.deb: XZ Max projection appears on the screen.
4. Generate a Slice Viewer of **the Untitled1.deb: XZ Max projection** by clicking on the **Slice Viewer** button on the Toolbar. The median slice number, approximately 34, will be displayed. Compare the axial deblurred result to the raw data set.

Tutorial for Batch Menu Item

This function can be launched directly from the **Deconvolution** menu, and selecting Batch Process. The Batch process is an alternative choice to starting the deconvolution process. When chosen, the Batch function "docks" the data set and their settings in a queue for processing at a later time.

Batch Process

➡ Click on **Batch Process** in the **File** menu.

Start

This function starts the deconvolution process for the batch files in the queue. Once the Batch process has started, you may cancel the processing of one or more of the files by pressing the Cancel button on the progress box. This cancellation will only apply to the file currently being processed and will not effect the previously processed files or the files yet to be processed.

Note: This Menu item will not become available unless there are image stacks in the queue

Batch Processing Form

This function allows you to navigate to a folder and select the files you would like to add to the Batch. Pressing the Explore button launches Windows Explorer. Once the file of interest is located, you have the option of clicking on the file and dragging it into the Batch queue or copying the file and pasting it into the Batch queue.

Cancel

This function closes the Batch feature without starting the processing on the files.

Clear List

This function allows you to remove all the files in the Batch queue.

Delete

This function allows you to remove the selected file from the Batch queue. To use this feature first select the file to be deleted; then press the **Delete** button.

Paste

This function allows you to paste a file that was copied into the Batch queue.

Help

This function launches the online Help files.

Adding Files to the Batch

To add files to a batch:

1. Select **Batch Process** from the **Deconvolution** menu.
2. Select the **Batch Processing** form from the **FILE** menu and navigate to the Tutorial Data directory.
3. Open the Batch folder and select the required files.

Note: There are three methods of adding a file to a Batch:

- With the file selected right click the mouse and choose **Copy**. Move your cursor to the Batch box and select **Paste**. The file appears as the first file in the queue.
- With the file selected click and drag the file over to the Batch box and drop it into the Batch queue.
- Open Pollen.deb in the **Leica Deblur and 3D Visualise** viewing window and select Start 3D Deconvolution from the Launch menu. Press the **Batch** button located at the bottom of the **Launch 3D Deconvolution** dialog. The file is automatically loaded into the Batch. To see that the file has been loaded, select **Batch Process** from the **Batch** menu. The file will be under the Image File Name list.

4. While the cursor is over one of the selected files, depress the left mouse button and drag the selected files into the Batch queue.

Note: Do not close the Batch Processing dialog. Continue on to the next section.

Accessing Tools

Note: You can access a variety of tools by right clicking the mouse to show a tools menu.

This menu allows you to copy and change the settings for the files in the Batch. The Tool menu items are: Copy Deconvolution Settings, Paste Deconvolution Settings, Change Settings, Change PSF File, Copy PSF File Settings, and Paste PSF File Settings. These features may also be accessed by moving your cursor over the files in the Batch and right clicking the mouse.

Copy Deconvolution Settings

This feature allows you to copy the setup and deconvolution parameters from one of the files in the Batch.

To copy deconvolution settings:

1. Select the file of interest from the Batch by clicking on it.
2. Select **Copy Deconvolution Settings** from the **Tool** menu. This automatically copies the file's parameters. If you paste the copied settings file into a text editor (for example, Windows Notepad) you will be able to view the actual settings for the deconvolution.

Paste Deconvolution Settings

This feature allows you to apply the setup and deconvolution parameters from one file into one or more files in the Batch. This feature is generally used when similar files are collected using the same microscope setup.

To paste deconvolution settings:

1. Select the file(s) from the Batch that you would like to copy the deconvolution parameters to. In this case, the PollenPA.deb file.
2. Select **Paste Deconvolution Settings** from the **Tool** menu. This automatically Pastes the Pollen.deb deconvolution parameters into the PollenPA.deb file.

Change Settings

This feature allows you to change the setup and deconvolution parameters for a file in the Batch.

To change the setup and deconvolution parameters for a file in the Batch:

1. Select the file from the Batch that you would like to change. For example, the PollenPA.deb file.
2. Select **Change Settings** by right clicking the mouse and choosing from the menu displayed. The **Launch 3D Deconvolution** dialog appears. Change the **Save Interval** setting to 20 and click **Update Batch**. This will update the settings in the Batch for the PollenPA.deb file.

Change PSF File

This feature allows you to change the PSF file used in a Non-blind deconvolution.

To change a PSF file:

1. Select the **PollenPA_psf.deb file** in the Batch file.
2. Select **Change PSF File** by right clicking the mouse and choosing from the menu displayed. The **Open** dialog appears. Navigate to the **Batch folder** and select **Pollen_psf.deb**. Click the **Open** button. The file will automatically replace the PollenPA_psf.deb.

Copy PSF File Settings

This feature allows you to copy the PSF File parameters from one of the files in the Batch.

To copy PSF file settings:

1. Select the **TOSPollen_psf.deb** in the Batch by clicking on it.
2. Select **Copy PSF File Settings** by right clicking the mouse and choosing from the menu displayed. This feature copies the file's parameters.

Note: If you were to paste this file into a text editor, you would be able to view the actual settings for the deconvolution.

Paste PSF File Settings

This feature allows you to apply the PSF parameters from one file into one or more files in the Batch. This feature is generally used when similar files are collected using the same microscope setup.

To paste PSF settings:

1. Select the **Pollen_psf.deb** file from the **Batch1 queue**.
2. Select **Copy PSF File Settings** by right clicking the mouse and choosing from the menu displayed.
3. Click on the **TOSpollen_psf.deb** file to make it active.
4. Select **Paste PSF File Settings** by right clicking the mouse and choosing from the menu displayed. This automatically Pastes the Pollen_psf.deb PSF parameters into the TOSPollen_psf.deb file.
5. Close all views before going on to the next tutorial.

Chapter Eleven: Publishing Results

Chapter Overview

This chapter contains the following topics:

- Introduction
- Setting up the Publishing Environment
- Printing Images
  - Selecting the Images to be Published
  - Setting Up the Print Image Environment
- Producing a Report
- Montaging Images
- Producing a Web Page
- Producing a Web Montage
- Making Movies

Introduction

You can publish the results of your experiment by:

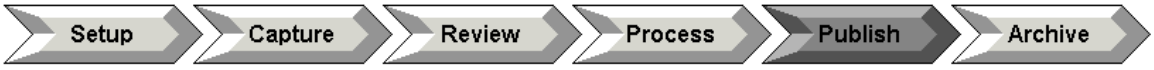
- Printing reports using the supplied Leica template that can also be edited to suit the requirements of the individual laboratory.
- Printing images with a choice of 1, 2 or 4 images per page.
- Printing a selection of images from a Z-stack or Time-lapse experiment. This is called Montage.
- Generating a report as a web page document.
- Making a movie from a set of images.

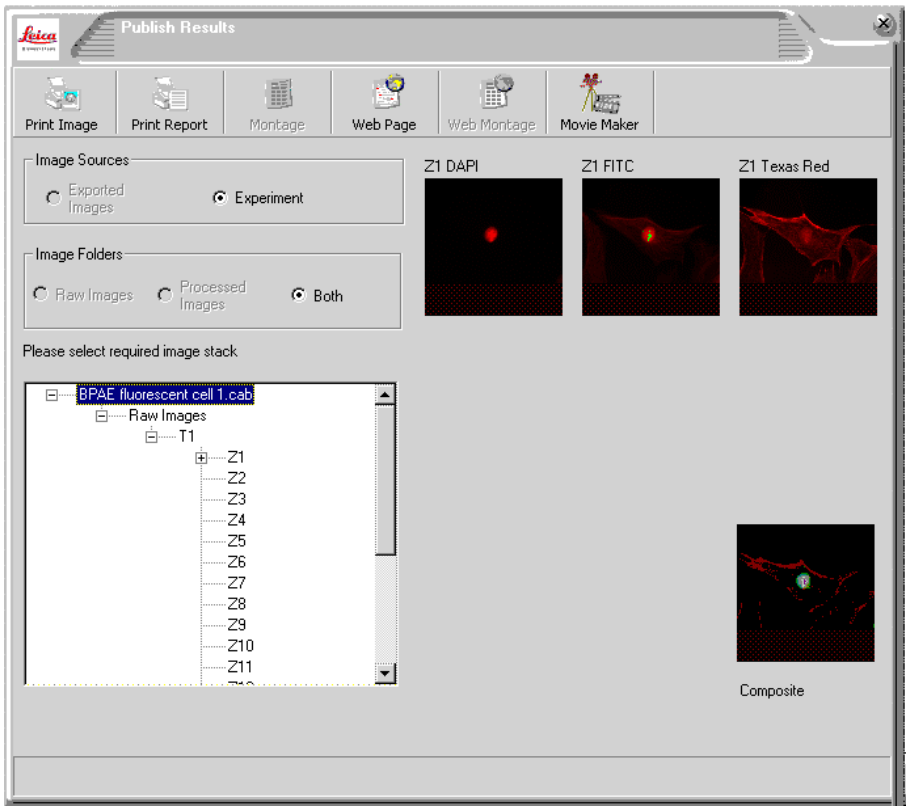
All options except the second one require Microsoft Word.

Leica FW4000 provides a bespoke Microsoft Word template for you to use in compiling your reports.

Setting up the Publishing Environment

➡ To publish the images you have captured, click **Publish** in the Experimental Setup process flow. The **Publish Results** dialog is displayed.





To produce a Montage report:

1. Select a set of images in the **Leica Image Gallery**.
2. Open the **Gallery** menu and click **Export Selected images to Publishing**. Close the Image Gallery.
3. Open the **Publish Results** dialog, to view all the images you selected for publishing.

➡ To show all the images used in your experiment, and produce a report of composite and component images, click **Experiment**.

Printing Images

Printing images is a two-stage process:

- Select the images you wish to print.
- Set up the print parameters.

Selecting the Images to be Published

You must select and prepare the composite or component images you wish to publish.

To prepare images for publishing:

1. Open the **Image Gallery** dialog.
2. Select the composite and component images to be published.

Refer to: *Chapter 6, Preparing Selected Images for Publishing* for more information.

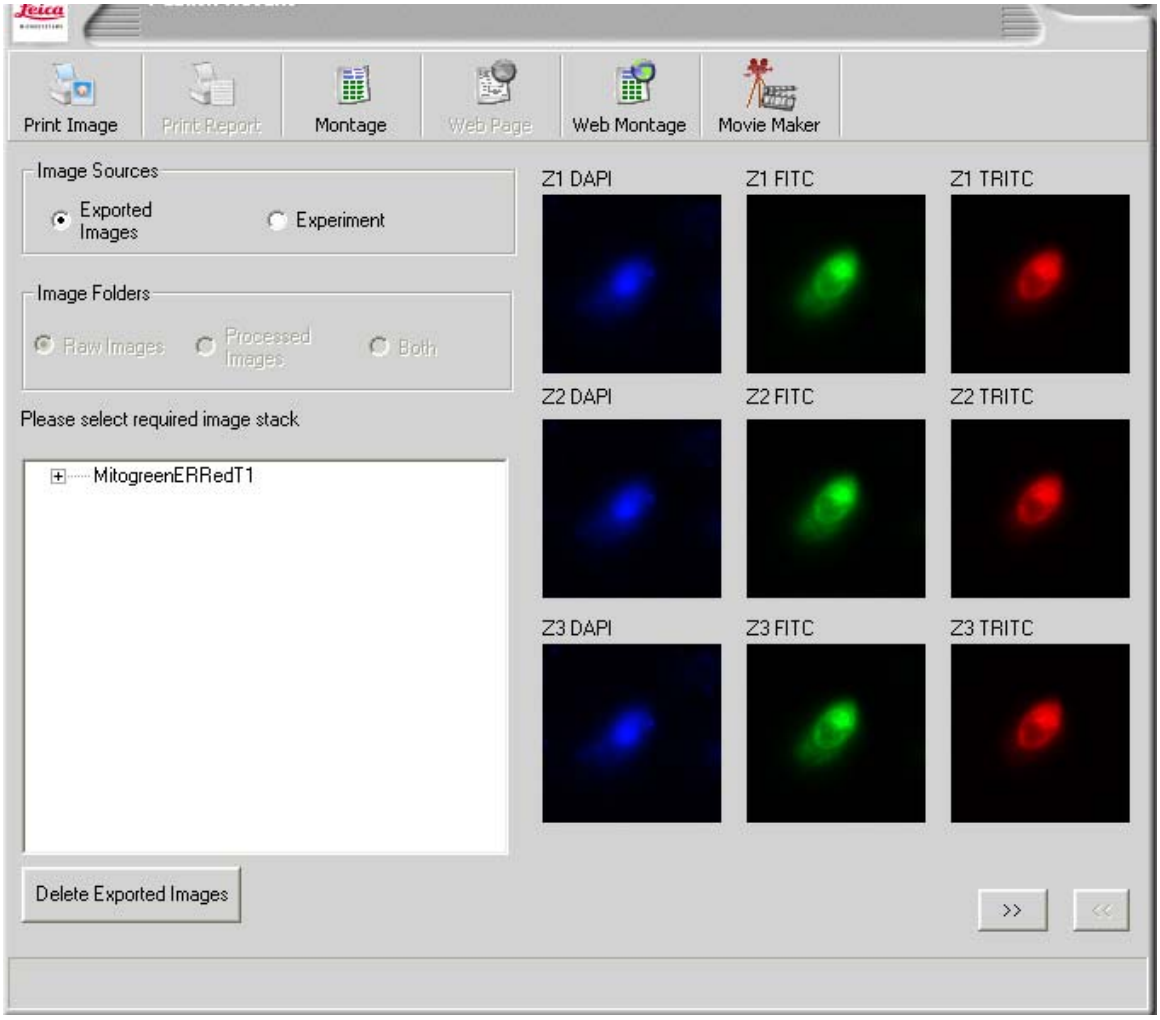
3. Open the **Gallery** menu and click **Export selected Images to Publishing.**

You can select the precise images you want to publish by expanding your experiment's image stack, and then dragging and dropping those images into the print image frame.

Click the appropriate radio button and choose whether to include **Processed Images** or **Raw Images** in the set of published images, or to use **Both**.

To select the images you wish to publish:

1. Expand the image stack for the selected experiment.

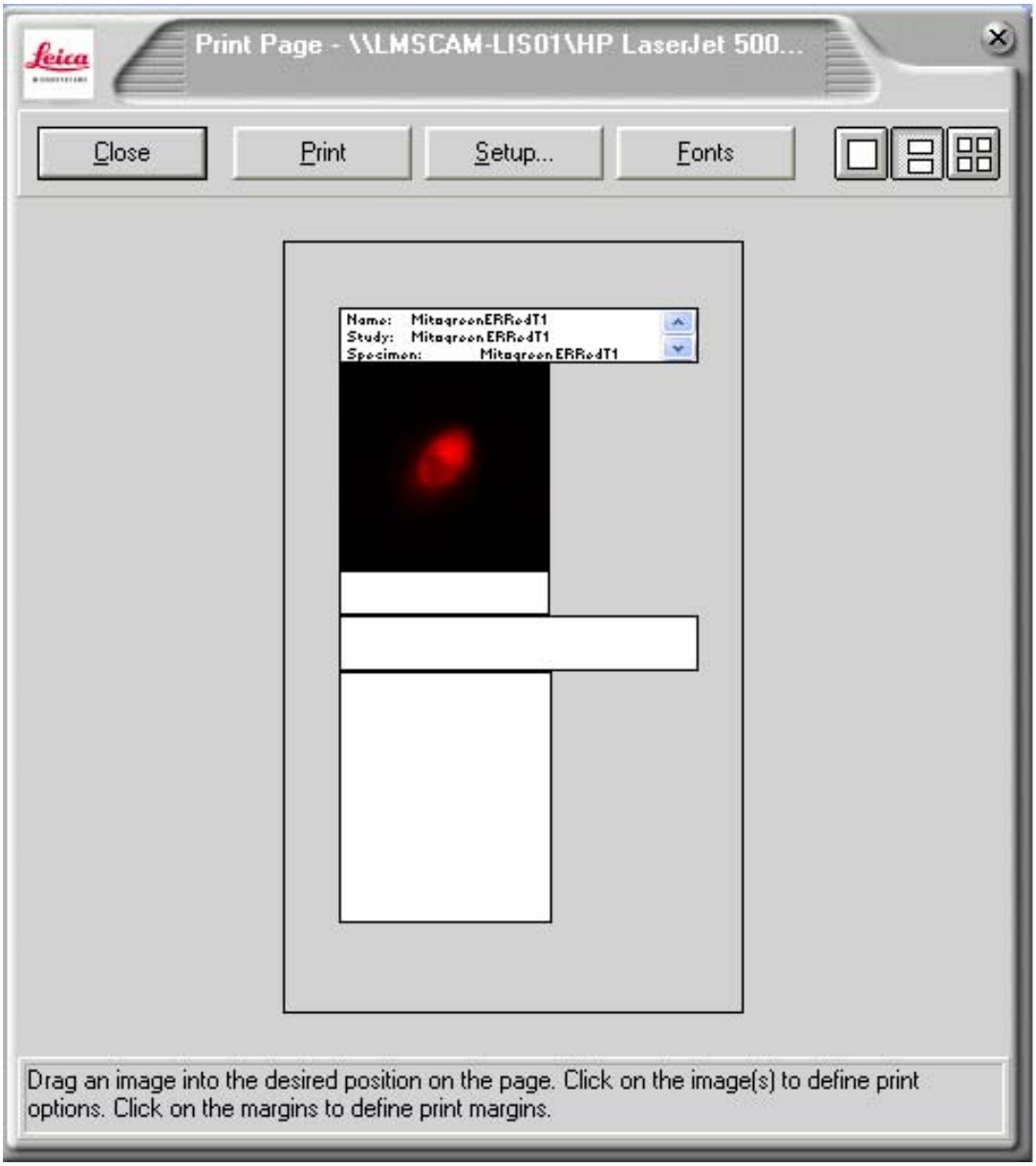


2. Click the reference to the image you wish to print. In the case above, TRITC, Z1 has been selected. This image is displayed in the viewer.

Setting Up the Print Image Environment

To print results:

1. Click **Print Image** in the **Publish Results** dialog main menu. This displays the **Print Page** dialog.

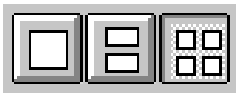

Print Page - \\LMSCAM-LIS01\HP LaserJet 500...
Close
Print
Setup...
Fonts
Name: MitogreenERRedT1
Study: MitogreenERRedT1
Specimen: MitogreenERRedT1
Drag an image into the desired position on the page. Click on the image(s) to define print options. Click on the margins to define print margins.

2. Drag the image into the **Print Page** dialog grid, and drop the image in the appropriate grid square.

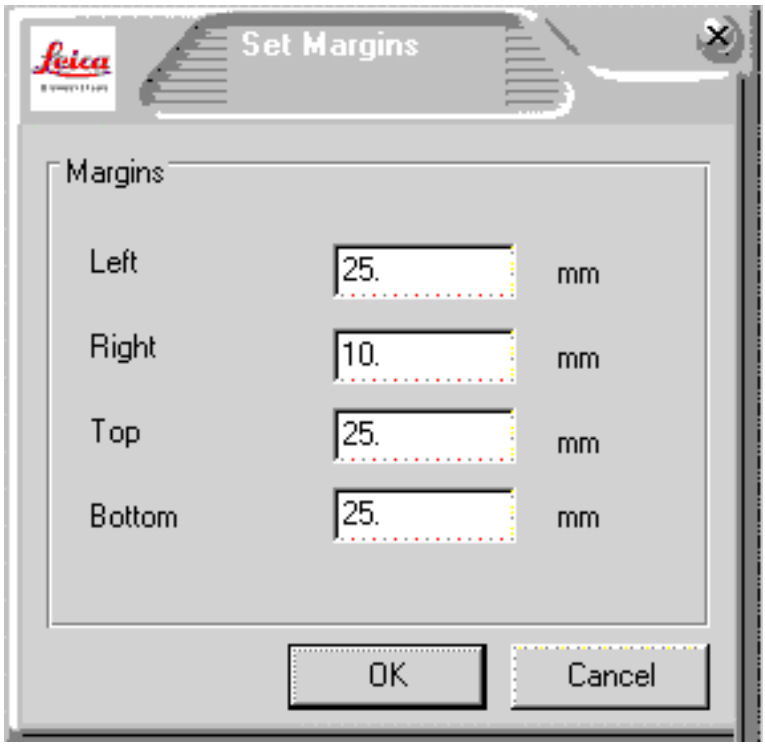
Note: To perform this drag and drop exercise you may need to move the Print dialog to one side of your screen to give you access to the image you

wish to drag and drop.

3. Repeat steps 2 and 3 until you have dragged and dropped all the images you wish to print.
4. At any time during the drag and drop activity you can change the print image grid by clicking the appropriate button.

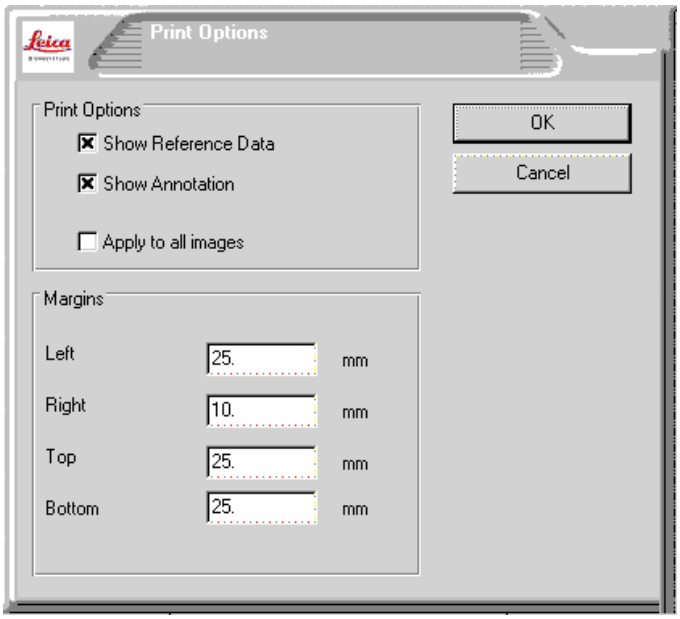


5. If you change the image grid from four images to either two or one image, then the images in the right bottom frames are removed as appropriate for the new grid layout.
6. To set the page margins, double-click the page outside the images in the **Print Page** dialog. This displays the Set Margins dialog.

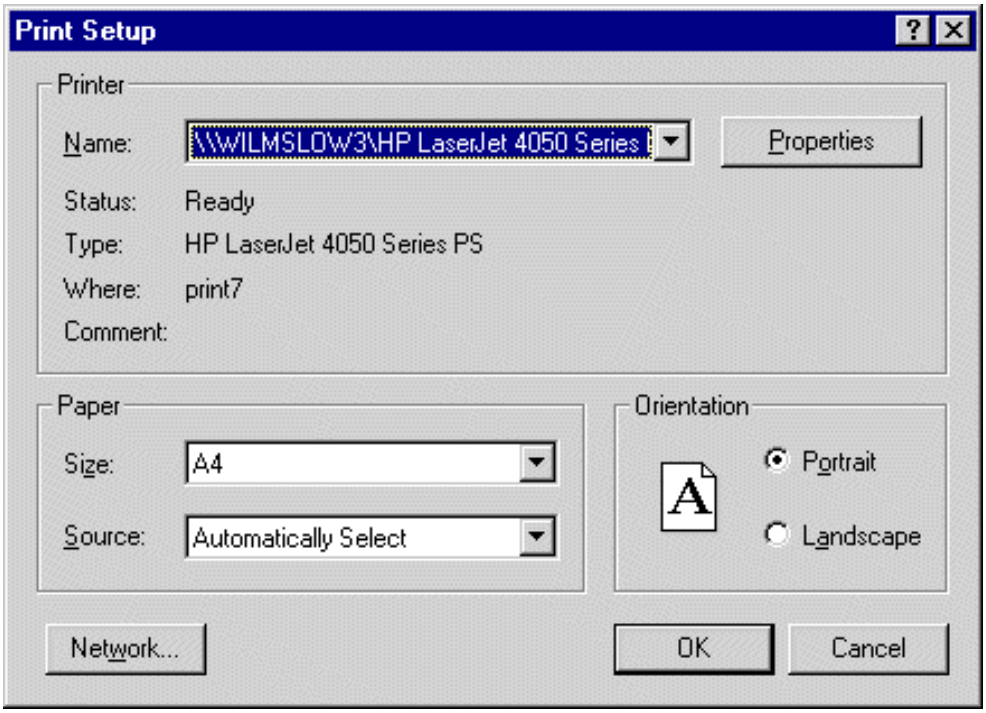


7. To specify the size of the margin, click in the appropriate text box and type the value in millimetres. A typical value would be in the region of 5 to 15 mm. To apply the new margin settings, click **OK**. Click **Cancel** to close the dialog without saving any changes.
8. Right-clicking on the image to be printed in the **Print Page** dialog displays **the Print Options** dialog. This allows you to toggle the **Show Annotation** and **Show Reference Data** facilities by clicking

the appropriate radio button.



9. Click **Setup** to display the **Printer Setup** dialog.



10. Select the printer type used by **Leica FW4000**. Select a printer and click **OK** to apply your choice.

Refer to: your *Microsoft Windows* documentation for information on using

the standard Printer Setup dialog and for using the Microsoft Windows Font dialog.

11. Click **Fonts** to change the font used to print the reference data. The standard Microsoft Windows **Font** dialog will be displayed.
12. Click **Print**, or click **Close** to close the **Print page** dialog together with the selected images in the frames.

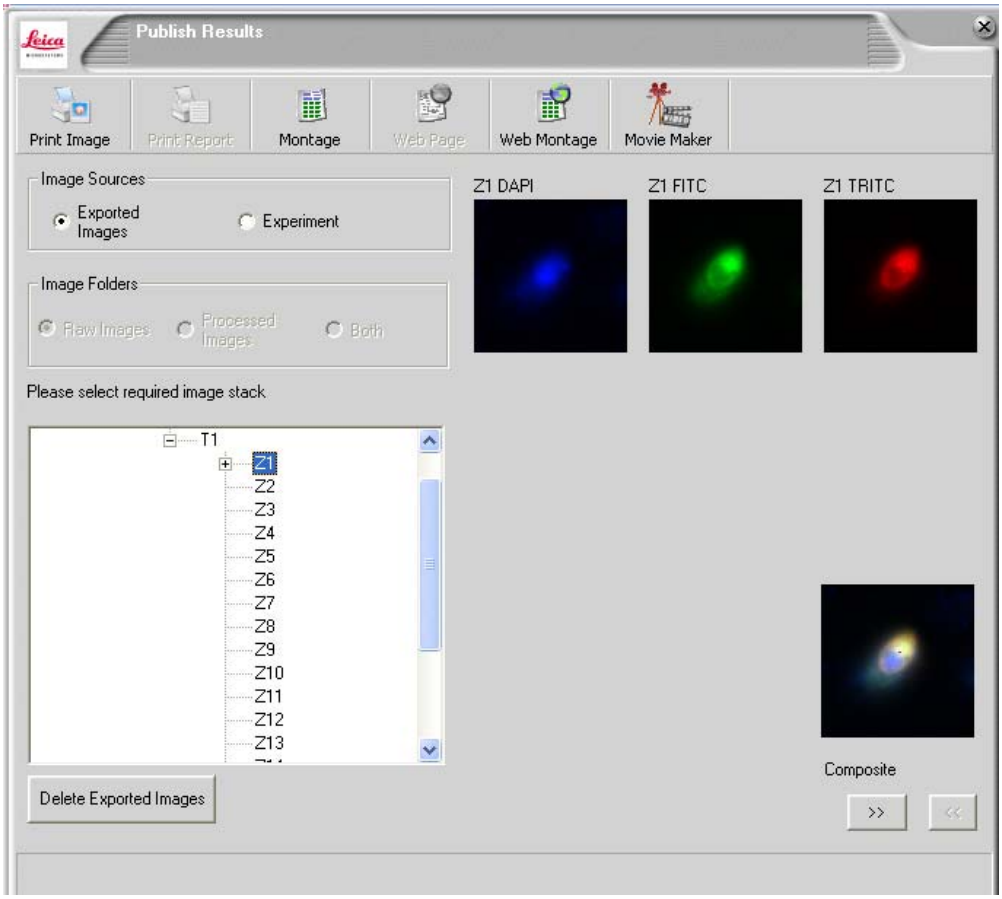
Producing a Report

You can select the results for the probes that you want to publish in your report.

➡ Click the appropriate radio button and decide whether to include **Raw Images** or **Processed Images**, or **Both** in the published images. Make sure Experiment is selected in the Image Sources options.

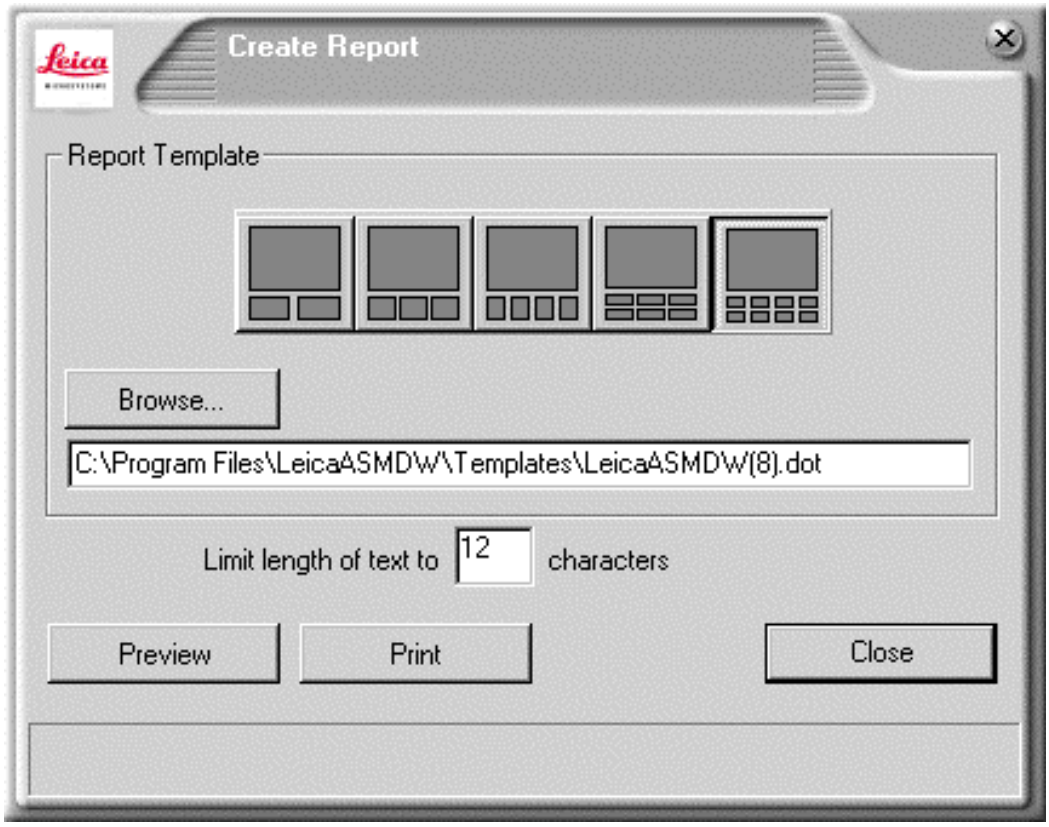
To produce a report:

1. Select the experiment radio button.
2. Expand the image stack for the experiment.
3. Select the desired probes at the Z plane level.



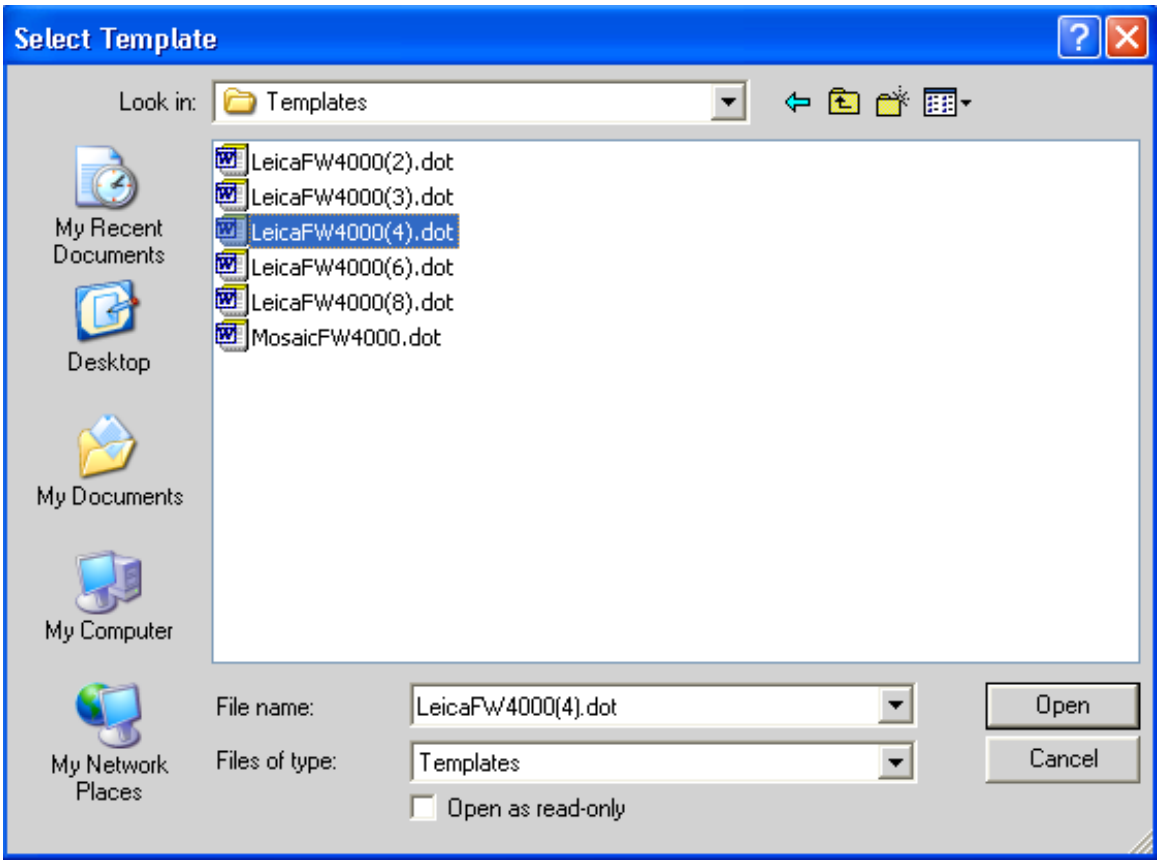
Note: Ensure that you highlight the lowest level of selection, that is Z1, Z2 Z3 etc, to display the full set of publishing buttons in the Publishing Gallery.

4. Click **Print Report** in the **Publish Gallery** dialog and display the **Create Report** dialog.



5. Select the appropriate report template for the number of probes you have used in your experiment by clicking one of the format buttons in the centre of the **Create Report** dialog.

Alternatively, to select the report format, click **Browse** to display the **Select Template** dialog. Select the appropriate template and click **Open**.



6. To preview your report click **Preview**.

7. Click **Print** to print your report, or click **Close** to close the **Create Report** dialog without printing your report.

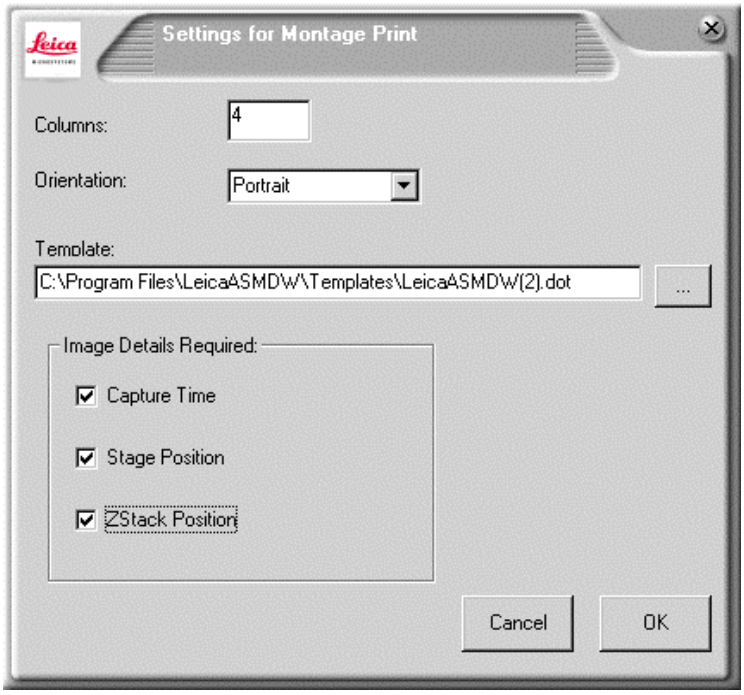
Montaging Images

The Montaging feature allows you to select Z and T series images from different image sets and publish them as a single set.

Note: To use this feature, you must have exported the required images from the gallery, and the Exported Images option must be selected in the Image Sources options.

To montage your selected images:

1. Click **Montage** from within the **Publish Results** dialog. This displays the **Settings for Montage Print** dialog.



2. Choose the number of columns to give the best layout for the images you are montaging.
3. Choose a page orientation of either portrait or landscape.
4. Click the ellipse and choose a template to use for laying out the montaged images.
5. Check the **Image Details Required** radio buttons, as required, to identify the image details to be published with each image.
6. Click **OK** to print the montage, or click **Cancel** to cancel the operation.

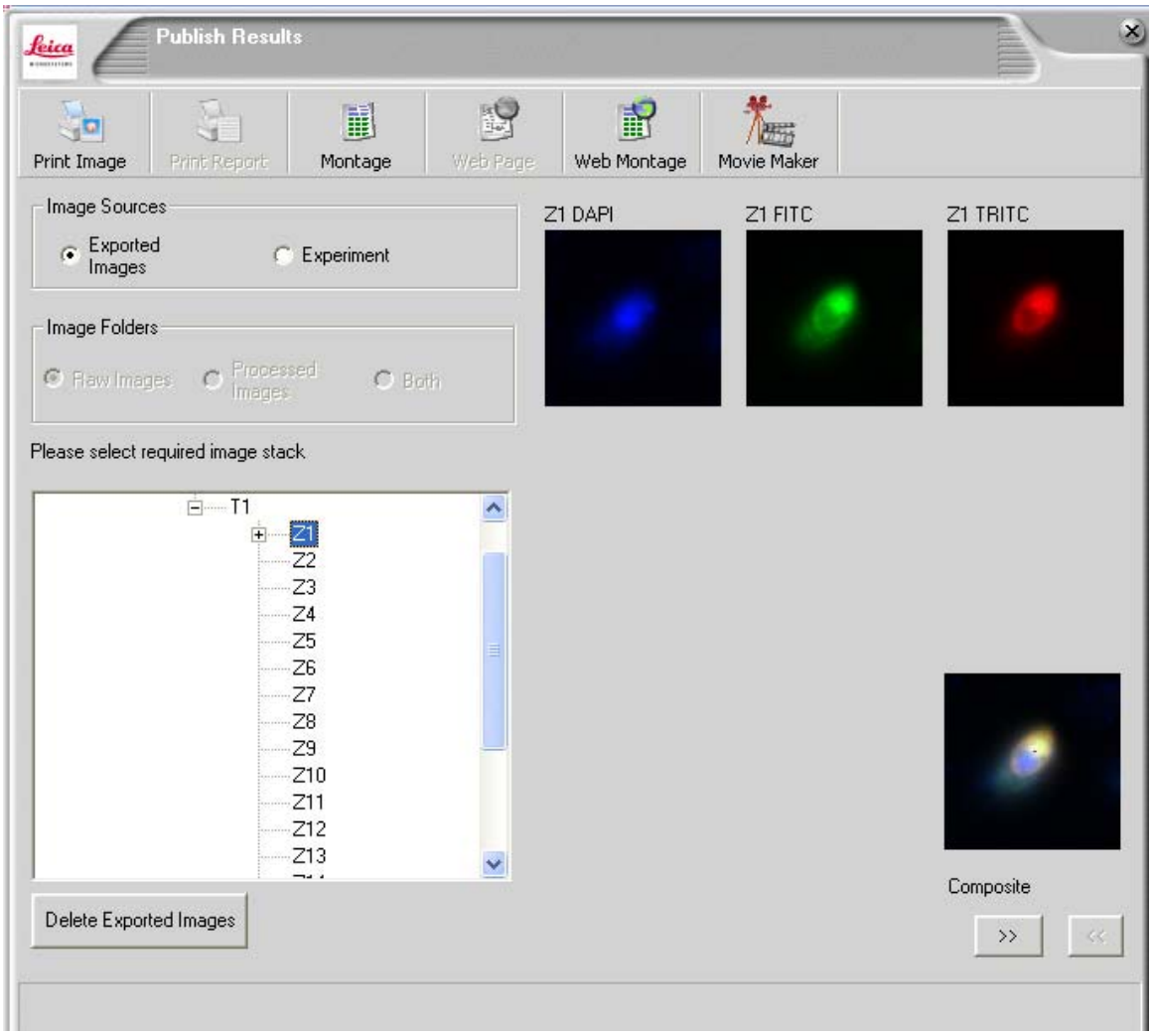
Producing a Web Page

You can select the experiment and the results for the probes that you want to publish in your web page.

➡ Click the appropriate radio button and decide whether to include **Raw Images** or **Processed Images**, or **Both** in the published images.

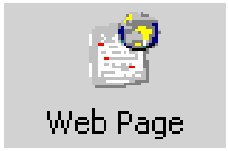
To produce a web page:

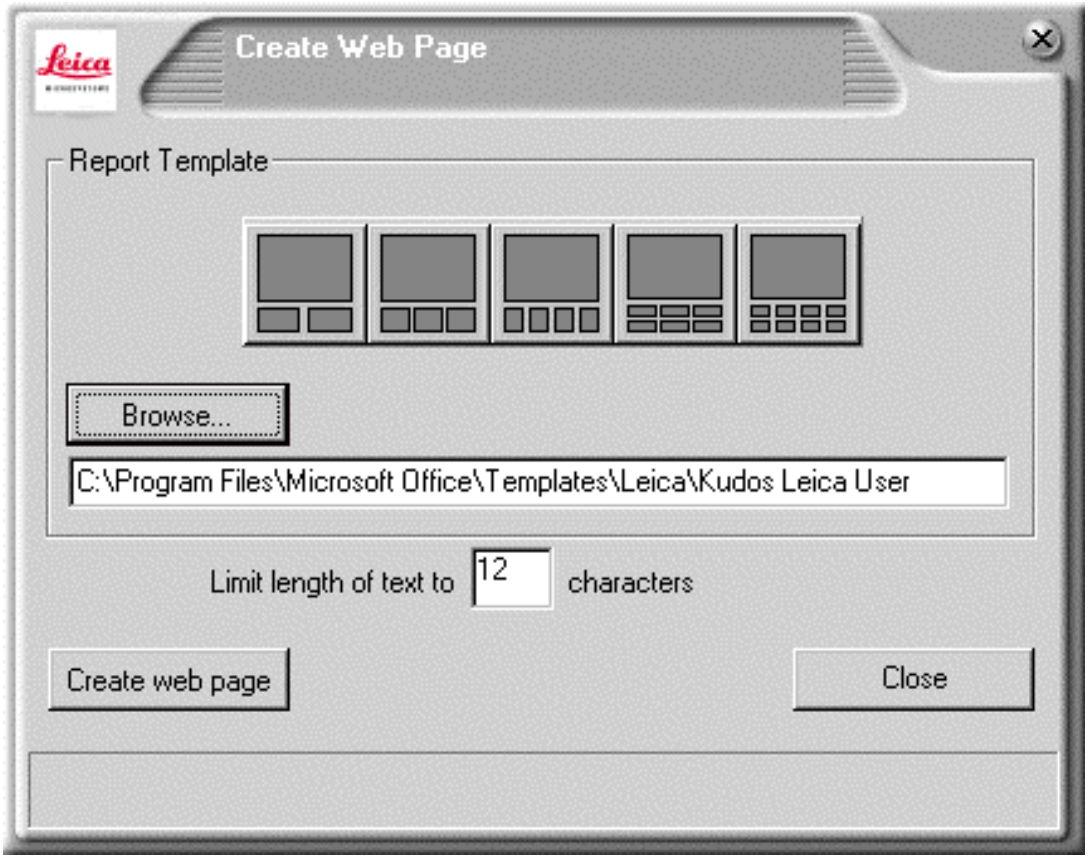
1. Select the experiment radio button
2. Expand the image stack for the selected experiment.
3. Select the desired probes at the Z plane level.



Note: Ensure that you highlight the lowest level of selection, that is Z1, Z2 or Z3, to display the full set of publishing buttons in the Publishing Gallery.

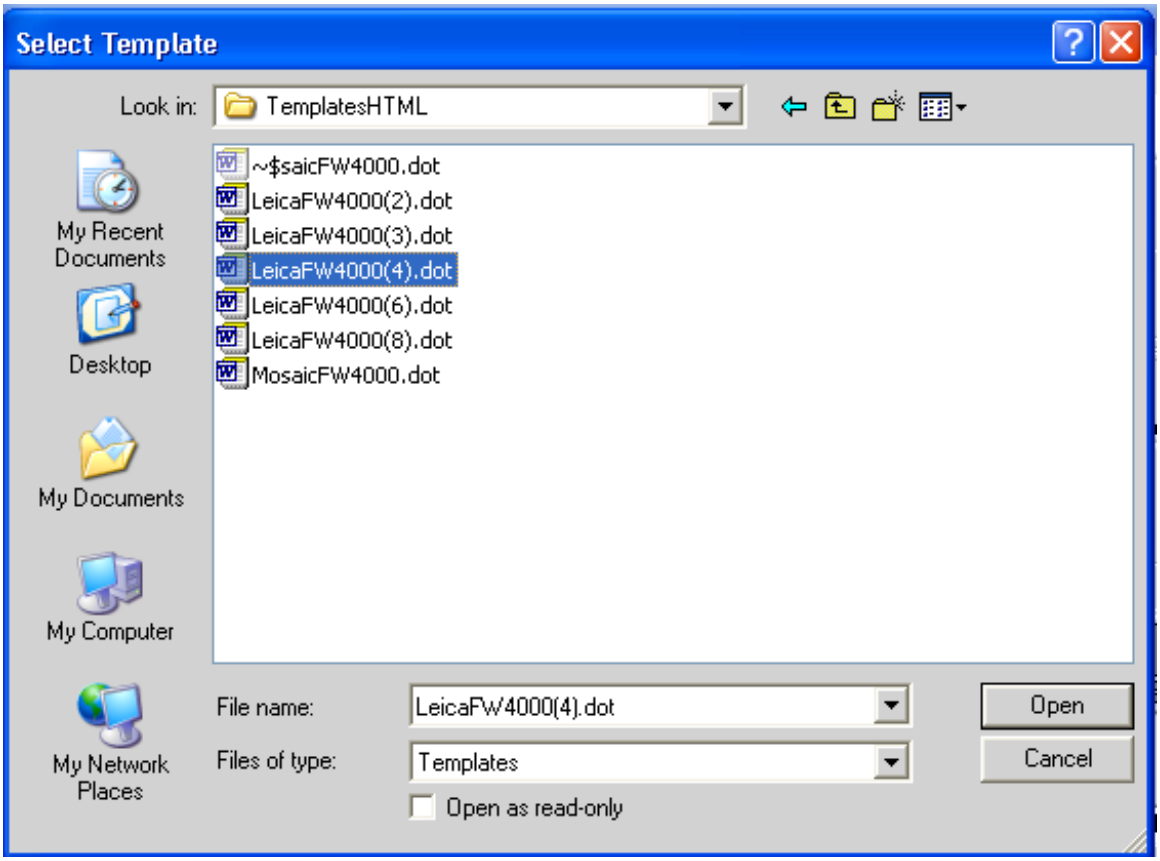
4. Click **Web Page** in the **Publish Gallery** dialog and display the **Create Web Page** dialog.





5. Select the appropriate report template for the number of probes you have used in your experiment by clicking one of the format buttons in the centre of the **Create Report** dialog.

➡ Alternatively, to select the report format, click **Browse** to display the **Select Template** dialog. Select the appropriate template and click **Open**.

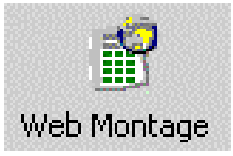


6. Click **Create web page** to create the web page, or click **Close** to close the **Create Web Page** dialog without creating the web page. You will be asked to specify a file name and location to which the web page is to be saved.

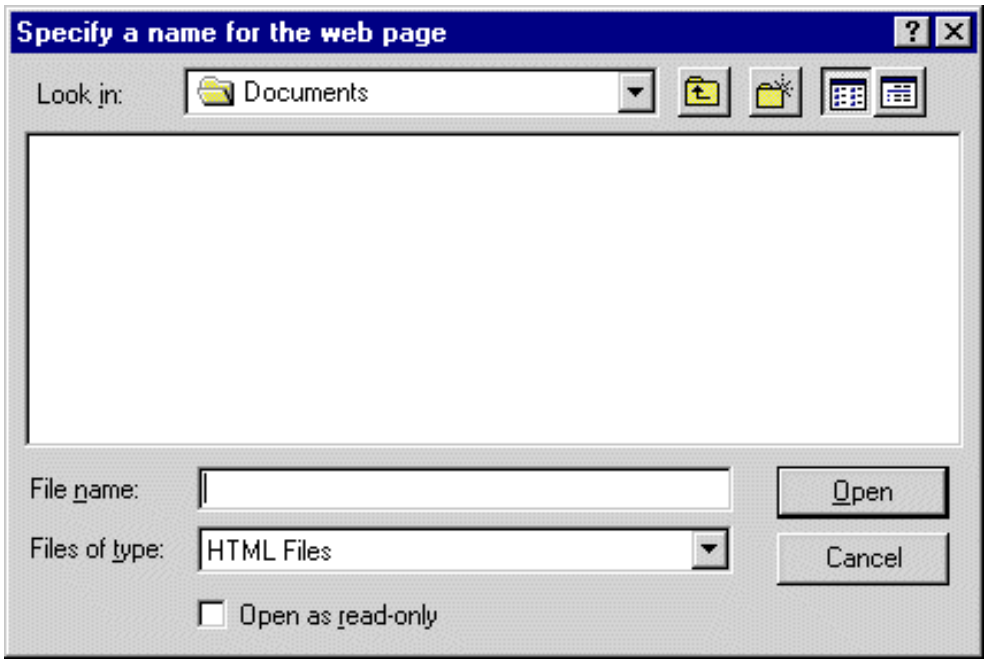
Producing a Web Montage

The web montaging feature allows you to select single composite and component images from different image sets and publish them as a web page montage.

To montage your selected images for web publishing:



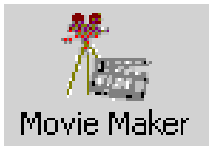
1. Click **Web Montage** from within the **Publish Results** dialog. This displays the **Specify a name for the web page** dialog.



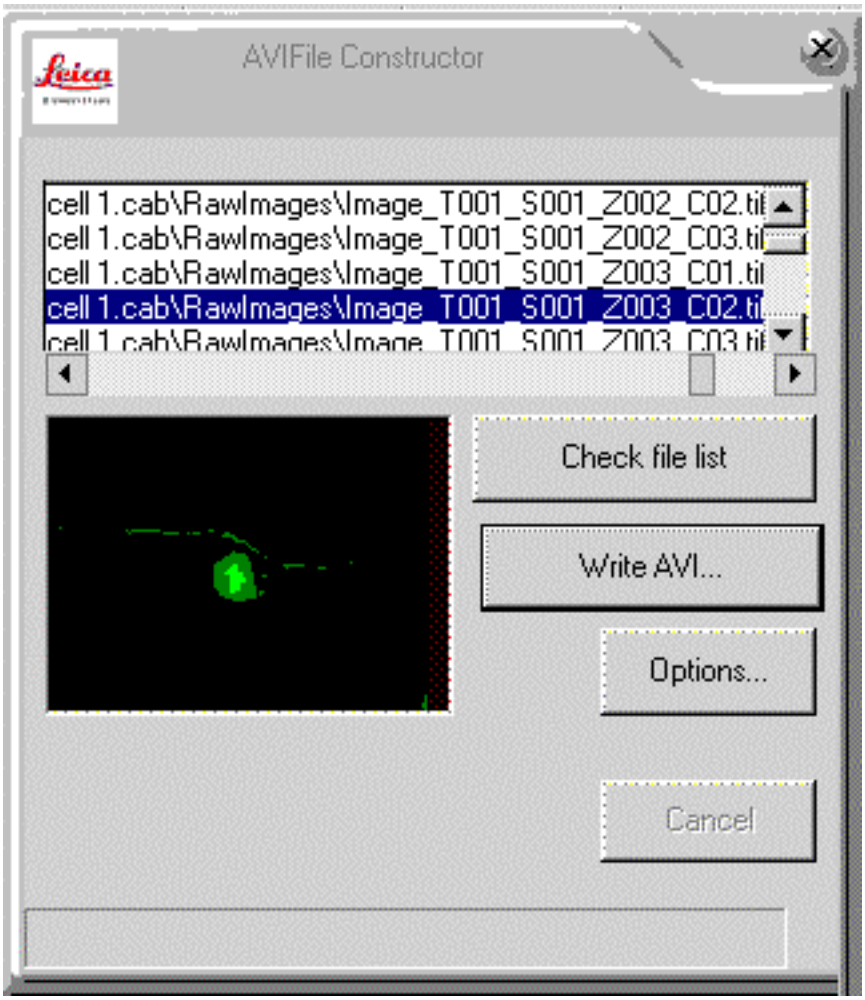
2. Type the name of the web page into the **File name** field.
3. Browse to an appropriate directory.
4. Click **Open**.

Making Movies

To make a movie that features your selected images:

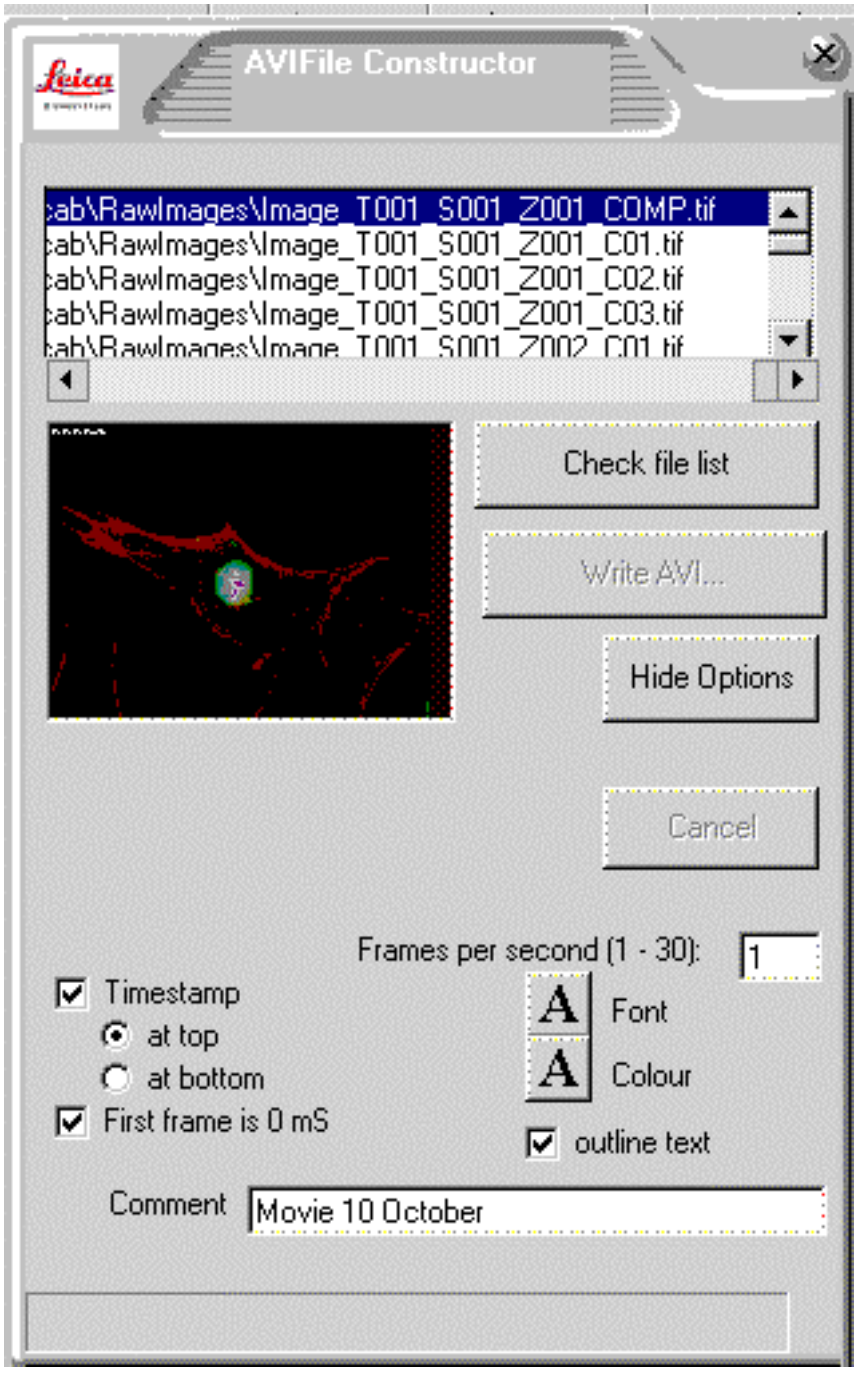


1. Click **Movie Maker** from within the **Publish Results** dialog. This displays the **AVI File Constructor** dialog.



2. Click **Check file list** to run through the files listed to check that the image files are of the same type and size. You can watch the filenames as they are automatically scrolled until the end of the list.
3. At the end of the check file run, **Check file list**, **Write AVI…** and **Options** buttons become active. You may interrupt the check file run by clicking **Cancel**.

4. Choose the number of frames per second by clicking **Options**, and typing a number in the range 1 to 30 in the box.

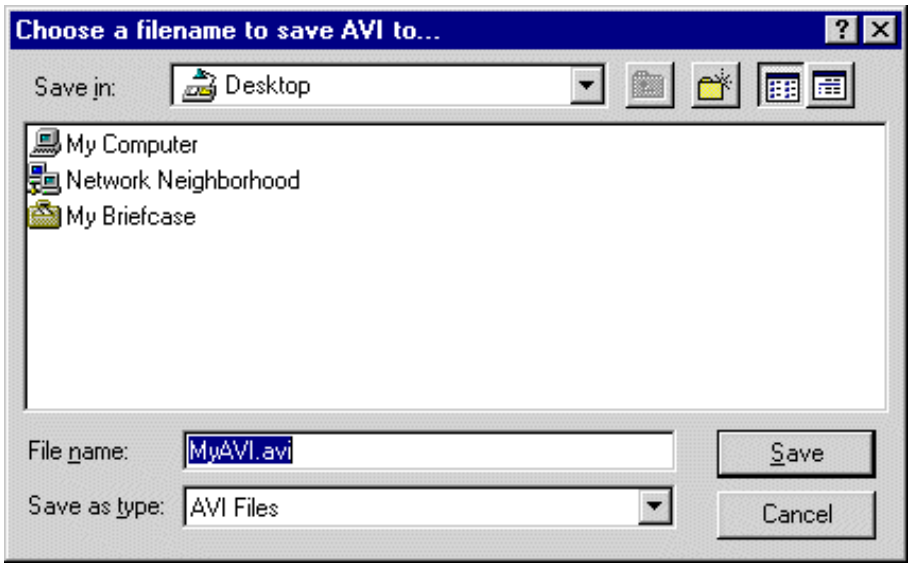


5. To timestamp each frame of your movie, click **Options**, click **Timestamp**, and choose the position of the timestamp, its font and colour, and define the first image to be time stamped.

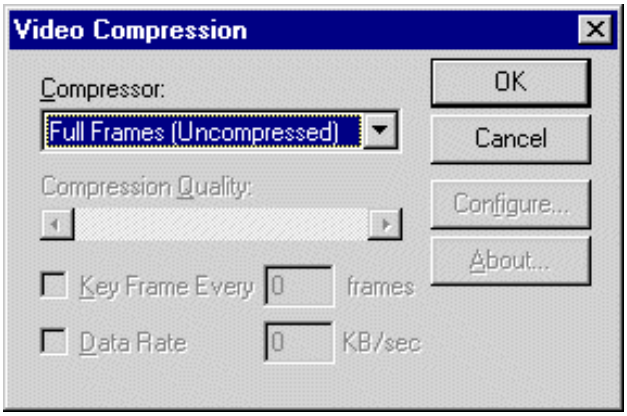
Note: You may define the first image as being acquired at time point zero, as opposed to the real time the image was acquired, and for each successive image to be stamped relative to the zero time point.

Note: Movies made in the movie maker from single channel images will appear in colour.

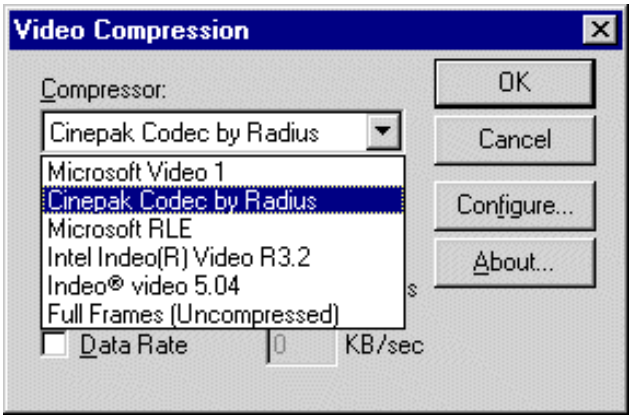
6. Click **Write AVI…** to display the **Choose a filename to save AVI to …** dialog. Save the movie with an appropriate filename in a directory of your choice.



7. Click **Save** to save the AVI, or click **Cancel** to cancel the operation. The **Video Compression** dialog is displayed.



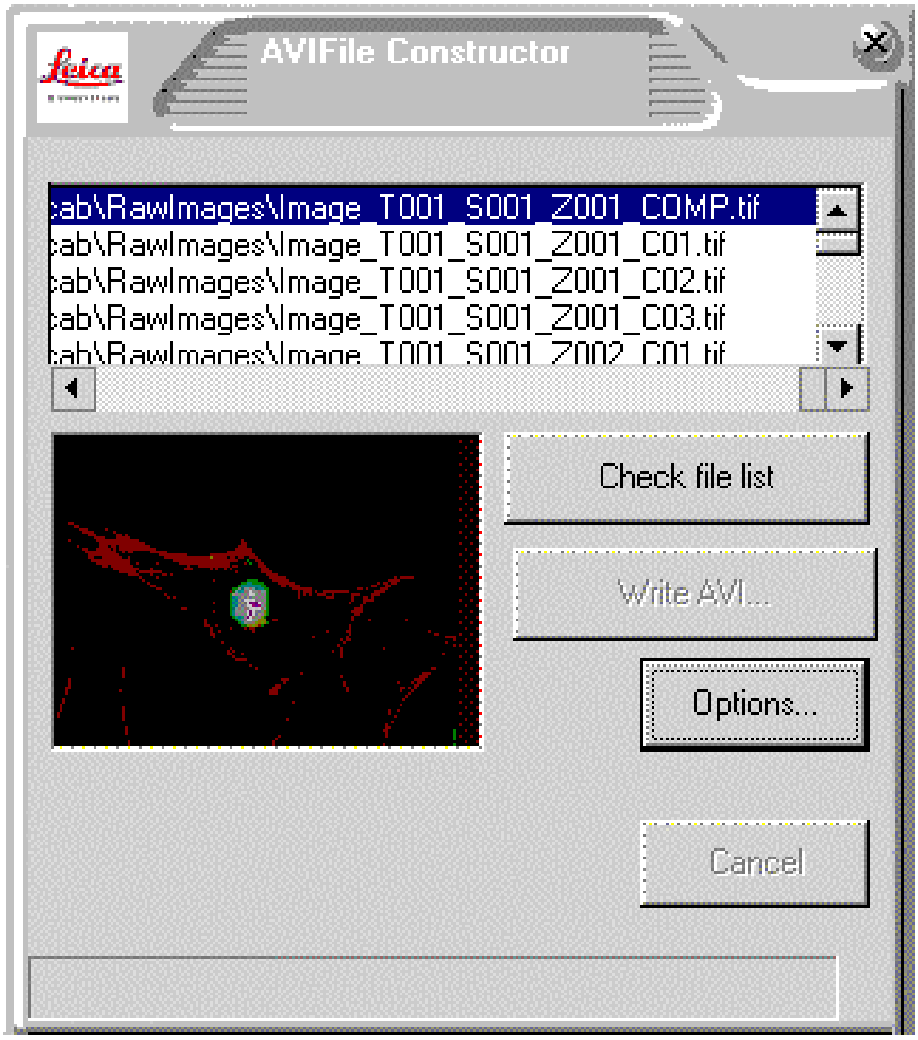
8. Choose the type of compression required for your movie system from the **Compressor** menu.



9. Move the slider to set the **Compression Quality**.
10. Set appropriate values for **KB/sec** and **Data Rate**.
11. Click **Configure** and set the configuration settings as required for your movie.

Note: The movie settings you can configure depend on the video compressor type you selected at step 6.

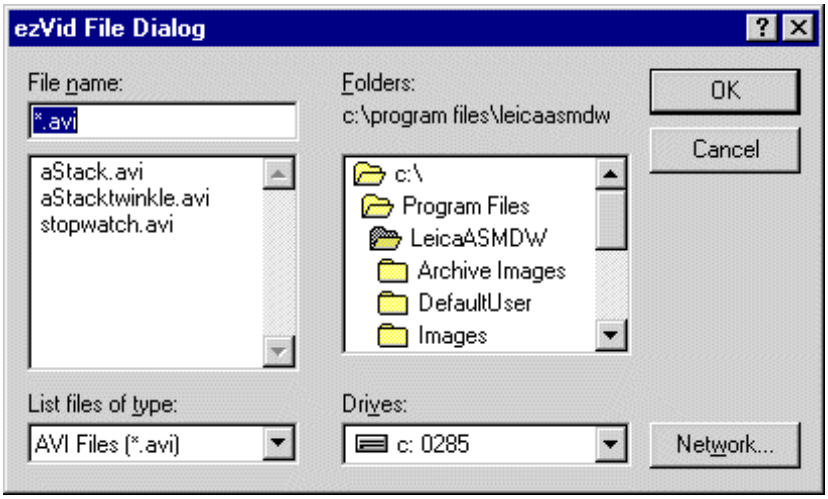
12. Click **OK**, or click **Cancel** to cancel the operation.
13. When you click **OK**, you display the **AVIFile Constructor** dialog and the real-time movie previewer.
14. You can watch a preview of the images, in the movie sequence, from start to finish in the AVIFile Constructor dialog.



15. You can see the **Video Clip Player** dialog.

16. Toggle the arrow button to turn the Movie Viewer tools on and off.

17. Click **OpenFile** to select the video file from the **ezVid File** dialog.
18. Browse and choose the movie to show, and click **OK**.



19. Set the parameters in the **Video Clip Player** dialog tools palette to the values you require to obtain the best effect when viewing the video of images from your experiment.
20. Use the double arrow buttons below the movie window to run the movie forwards and backwards. You can stop the movie at any time by clicking the **Stop** button (this is the square button in the centre of these controls). Also, you can step through the movie, one frame at a time, by using the single arrow buttons.
21. Click **Exit** to close the **Video Clip Player** dialog.
22. Click the button (a cross) in the top left corner of the **AVIFile Constructor** dialog.

Chapter Twelve: Archiving your Experiments

Chapter Overview

This chapter contains the following topics:

- Introduction
- Archiving Experiments
  - Selecting Experiments in Archive Using Properties
  - Archiving Experiments
- Retrieving Experiments from Archive
- Importing Tiff Images into Leica FW4000
- Deleting experiments

Introduction

During the course of setting up, performing and manipulating images for your experiment you may accumulate large amounts of data. When colleagues also perform experiments, they will accumulate large amounts of data. The combined effect of many people performing experiments means that huge amounts of data are accumulated and reside in your laboratory's filestore. To ensure that old experiments do not unnecessarily occupy (and fill) filestore you are strongly recommended to archive these experiments to an appropriate medium.

Archiving manages your experiments by using two tools available through the **Archive Experiments** dialog:

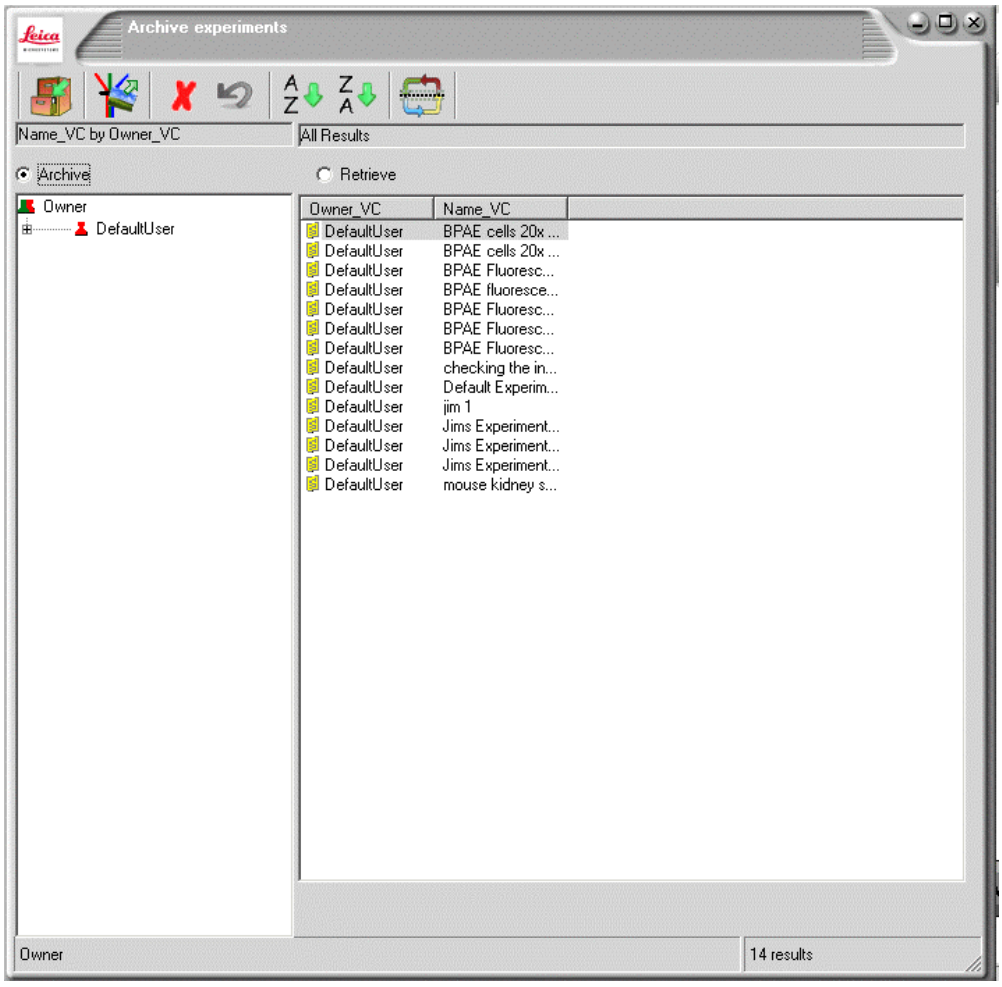
- Archive
- Retrieve

Archive stores an experiment on a storage medium but keeps a record in the database as to where you stored it. You use Retrieve to quickly get the experiment back, as the archiving system automatically requests the return of an experiment from the location where it is stored.

The other options available through the **Archive Experiments** dialog allow you to sort and select experiments so you can easily find the experiment you wish to archive or retrieve.

Archiving Experiments

➡ To archive your experiments click **Archive** in the Experimental Setup process flow. The **Archive experiments** dialog is displayed, and the archiving tools are ready for you to use. Check the **Archive** radio button.



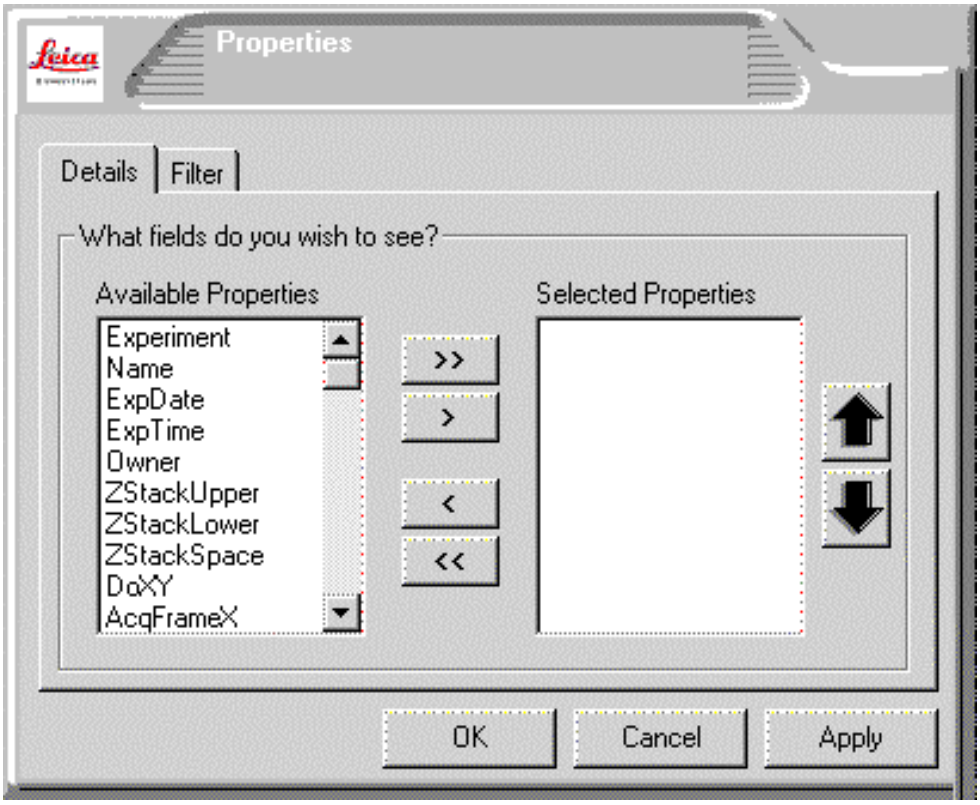
➡ To sort records in an ascending or descending order, highlight the records to be sorted and click the appropriate button.

➡ To exit from this stage in the Environmental Setup process, click the **x** button in the top right corner of the **Archive Experiments** dialog.

Selecting Experiments in Archive Using Properties

The **Properties** dialog allows you to select data and allow experiments to be found in the Lab Book by using experimental properties.

➡ To identify and set up the property profile for experiments to be archived, click on the **Properties** button. The **Properties** dialog is displayed.

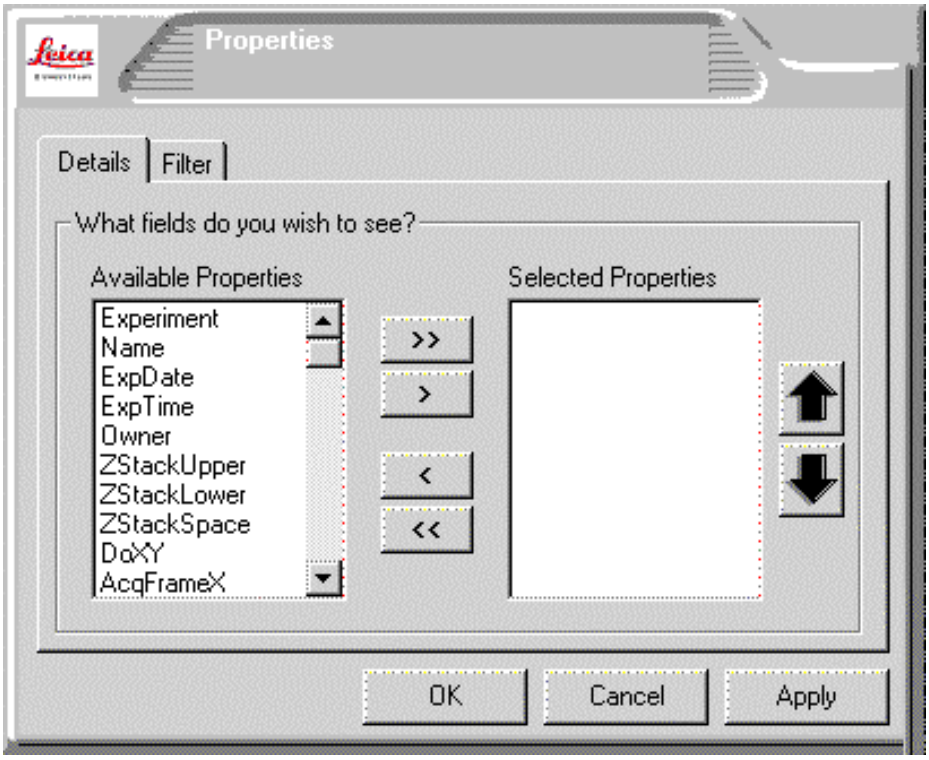


Details Tab

The **Details** tab allows you to select experimental properties that will appear in the archive window.

To build up the property details profile for an experiment to be archived:

1. Select the **Details** tab from the **Properties** dialog.



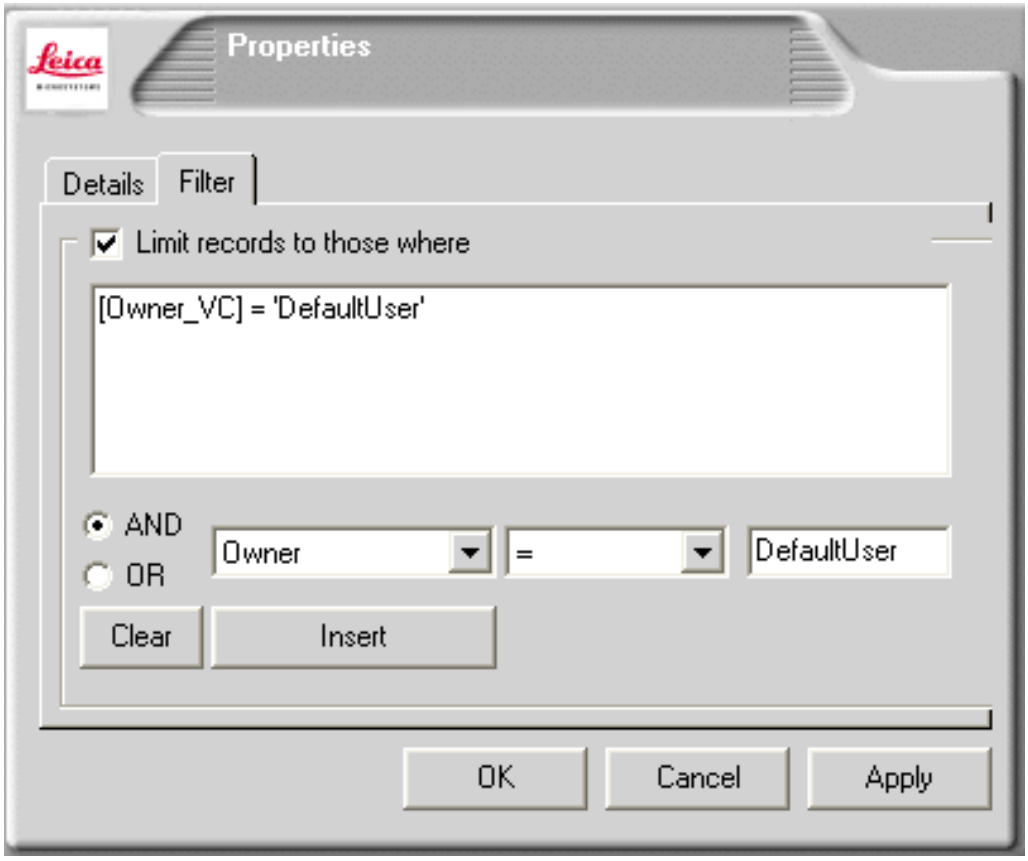
2. Build up a set of selected properties by highlighting a property in the **Available Properties** list, and clicking the single **right arrow** button. The highlighted result moves into the **Selected Properties** list. Repeat this action until you have selected and moved the required properties into the **Selected Properties** list.
3. If there are any results in the **Selected Properties** list in error, you can return those results to the **Available Properties** list by clicking the single **left arrow** button.
4. If you wish to move all properties between the two lists, click the double **right** or **left arrow** button.
5. When you are satisfied with your choice of selected properties, click the **Filter** tab.

Filter Tab

The **Filter** tab allows you to restrict the number of experiments displayed in the archive dialog using search criteria.

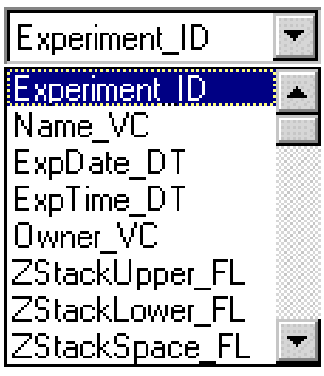
To build up the filter profile for an experiment to be archived:

1. Select the **Filter** tab from the **Properties** dialog.

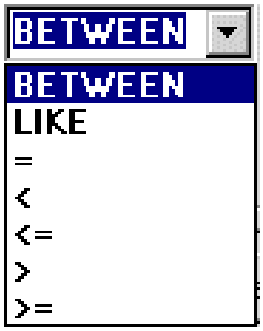


2. Check the Limit records to those where box.

3. Click the **Filter** menu arrow and click the required filter.
   In some cases, for example when you choose either **ExpDate_DT** or **ExpTime_DT,** you can further delineate your data by supplying a date or time on the right boxes.



4. Click the **Function** menu arrow and click the required function.

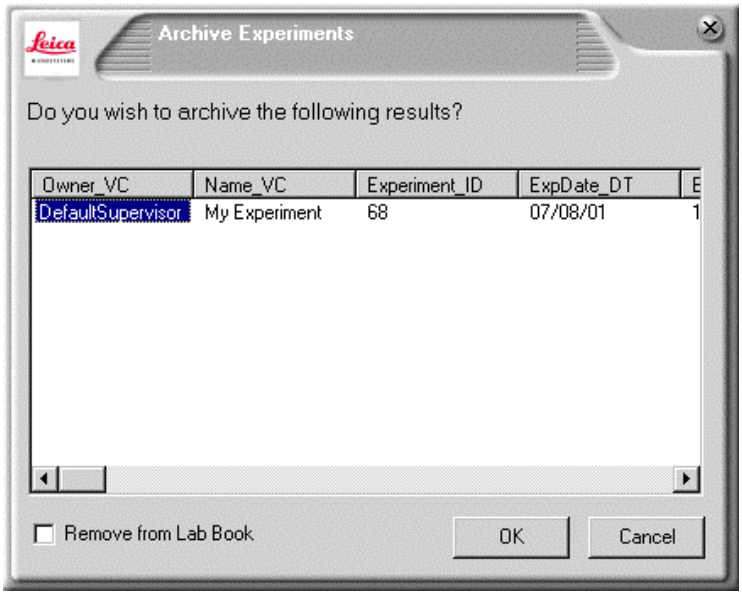


5. Click **Apply** to apply the data profile without closing the dialog. If you are dissatisfied with the profile, repeat steps 2 to 11 until you are satisfied. You can apply a more complex filter using the AND or OR radio buttons. However, do not use both AND and OR because the results will not be as expected.
6. Click **OK** to accept the total data profiles, or click **Cancel** to cancel the operation

Archiving Experiments

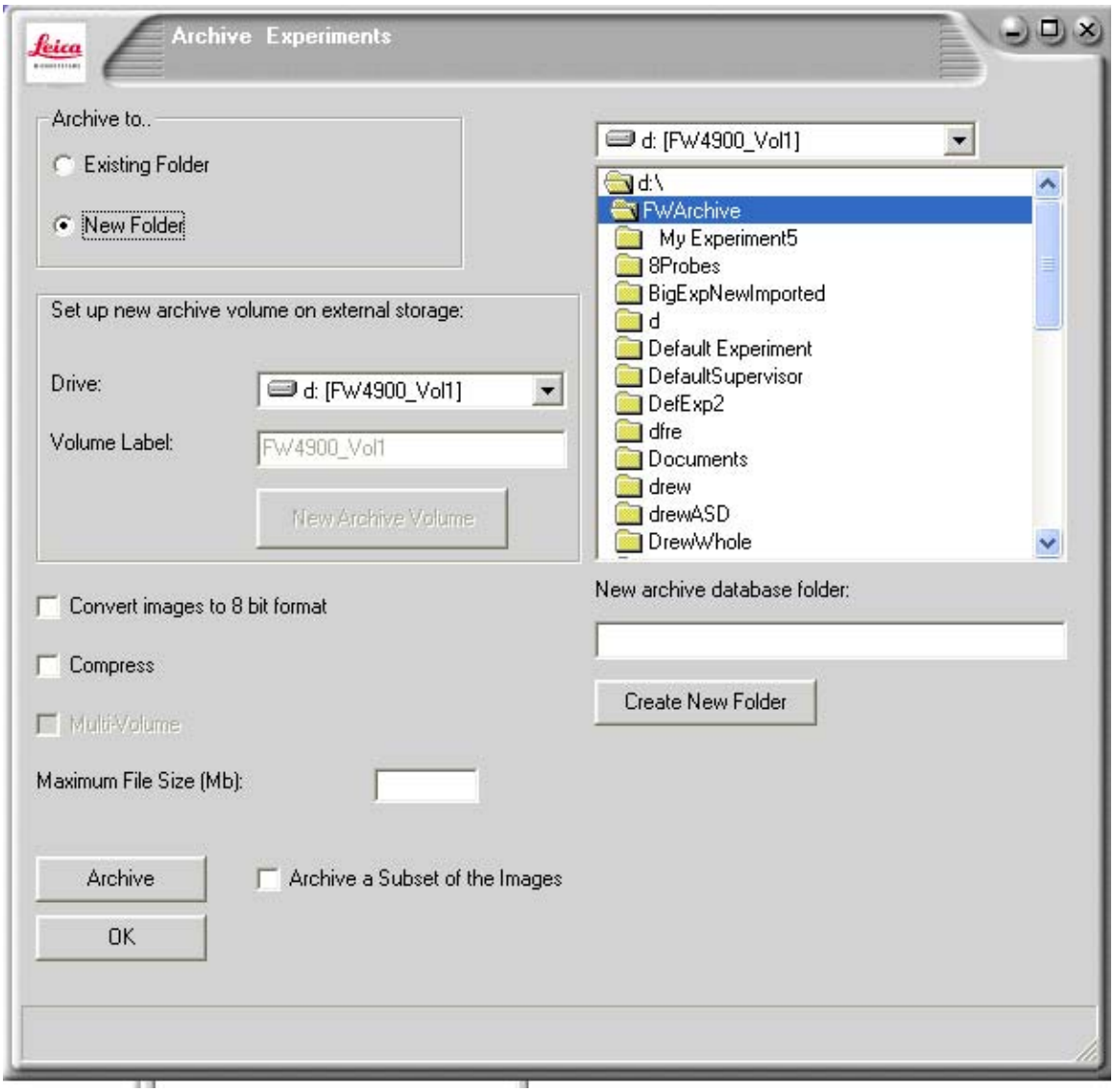
To archive an experiment:

1. Highlight the experiment to be archived.
2. Click the **Archive** button. The **Archive Experiments** dialog is displayed.



3. If you wish to remove the data from the laboratory workbook, check the **Remove from Lab Book** box.

4. Click **OK** to display the **Assign Archive Database** dialog.



5. Check the **Existing Folder** radio button to archive the experiment to the existing folder. You will need to click **Yes** to the dialog that asks you to confirm whether or not to overwrite the existing experiment.
6. Check the **New Folder** dialog radio button to archive the experiment to a new folder. The **Archive Experiments** dialog shown above is displayed..

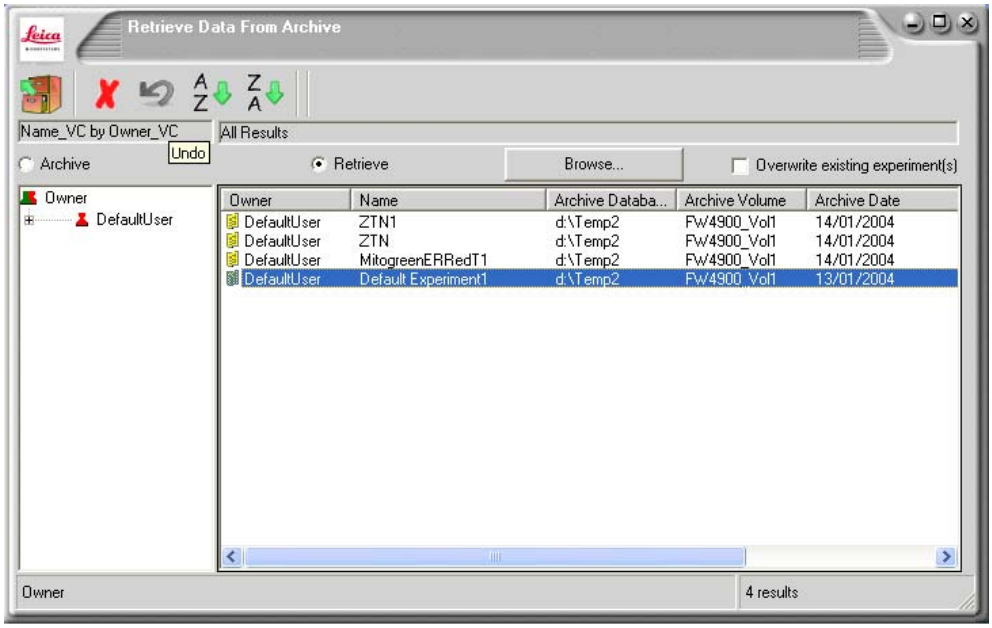
7. Browse the filestore directory hierarchy to select the directory in which to archive your experiment.

8. Type the name of the new folder, in the **Create New Folder** field, that will contain your archived experiment, and press Create New Folder.
9. Click **Archive**.
10. Optionally, you can use the check boxes provided to specify additional options:
    - Save images as 8 bit in the archive, or in compressed format.
    - Save large experiments to multiple volumes of a removable storage device.
    - You may wish to archive only a subset of the images, in which case a dialog is displayed, similar to the Select Required Images dialog in the gallery, to enable you to specify which images are to be saved.
11. Click **OK** to close the **Archive Experiments** dialog.

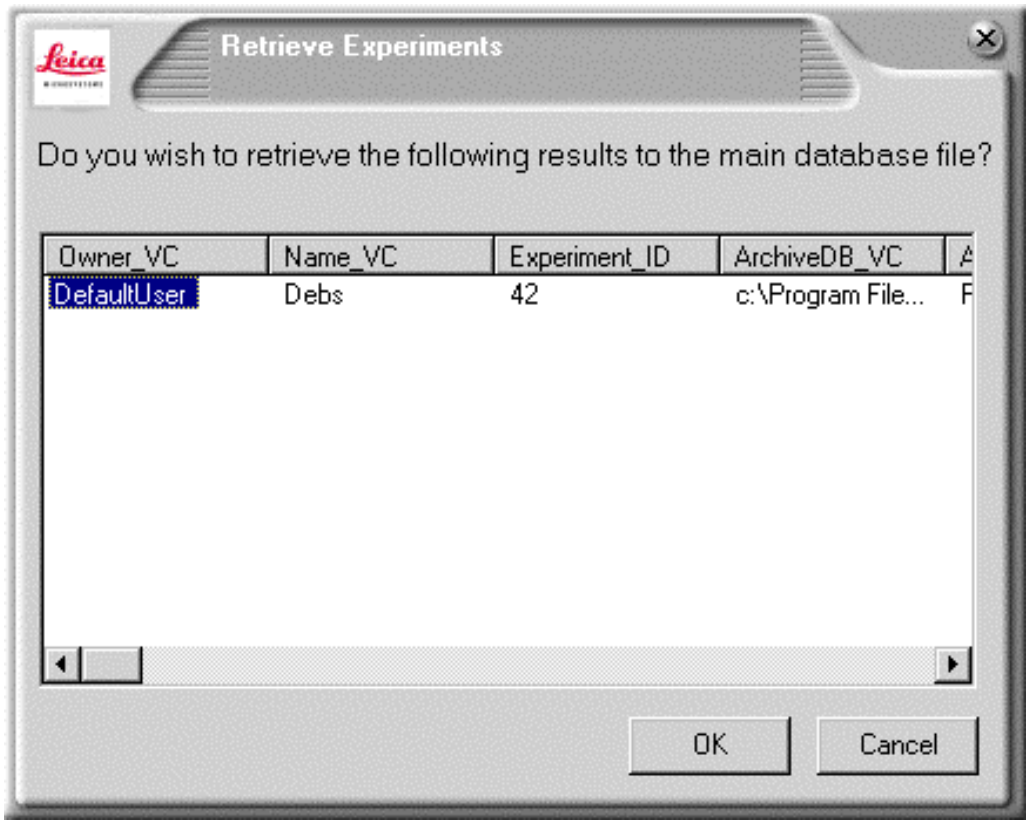
Retrieving Experiments from Archive

To retrieve an experiment from archive:

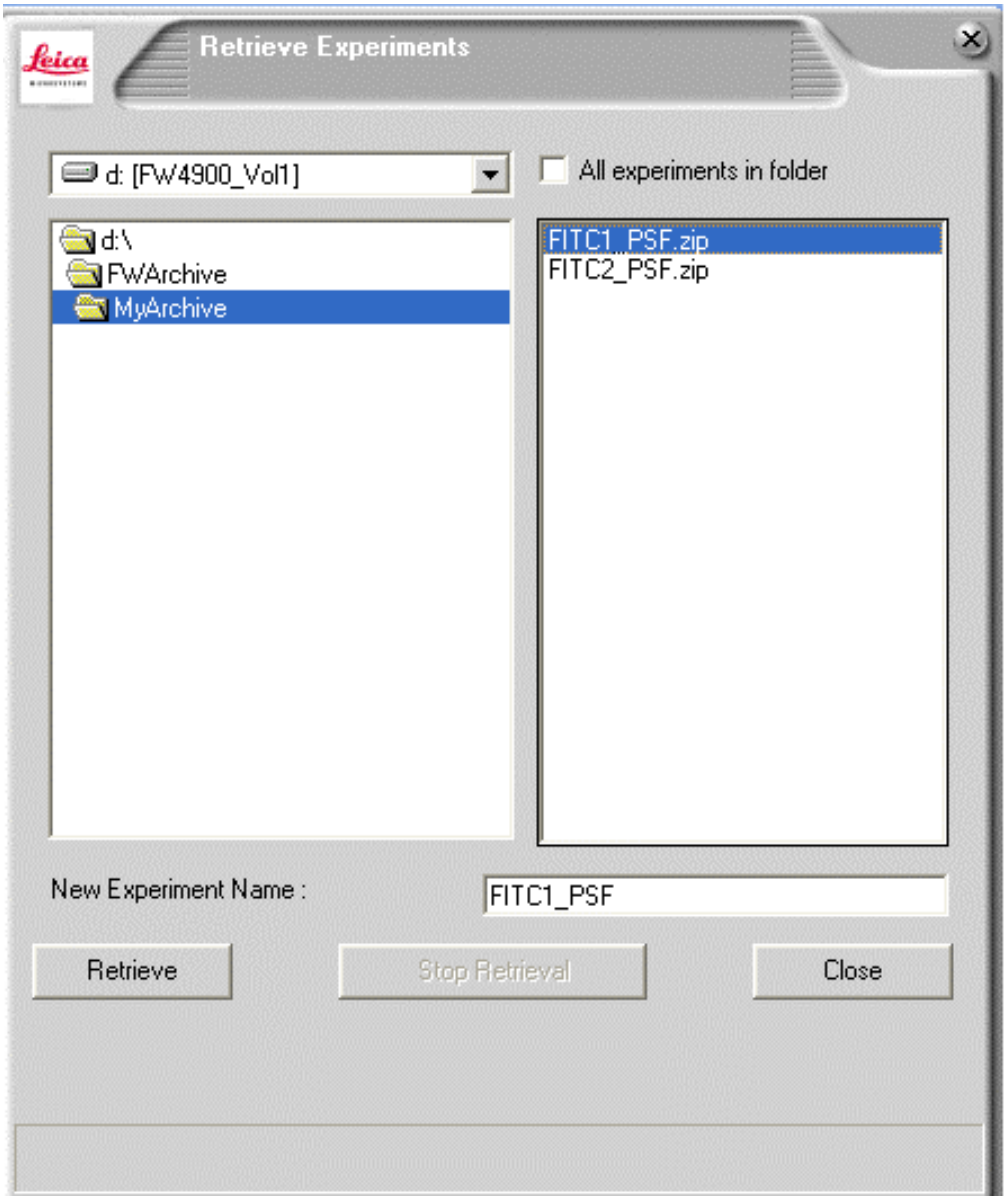
1. Click the **Retrieve** radio button. The **Retrieve Data from Archive** dialog is displayed.



2. If the experiment you wish to retrieve is displayed in the list, highlight the experiment you wish to retrieve.
3. Click the **Retrieve from Archive** button. The **Retrieve Experiments** dialog is displayed.



4. Click **OK** to retrieve the experiment, or click **Cancel** to cancel the operation.
5. If the experiment you want is not in the list, select the Browse button to browse to the location of the experiment.



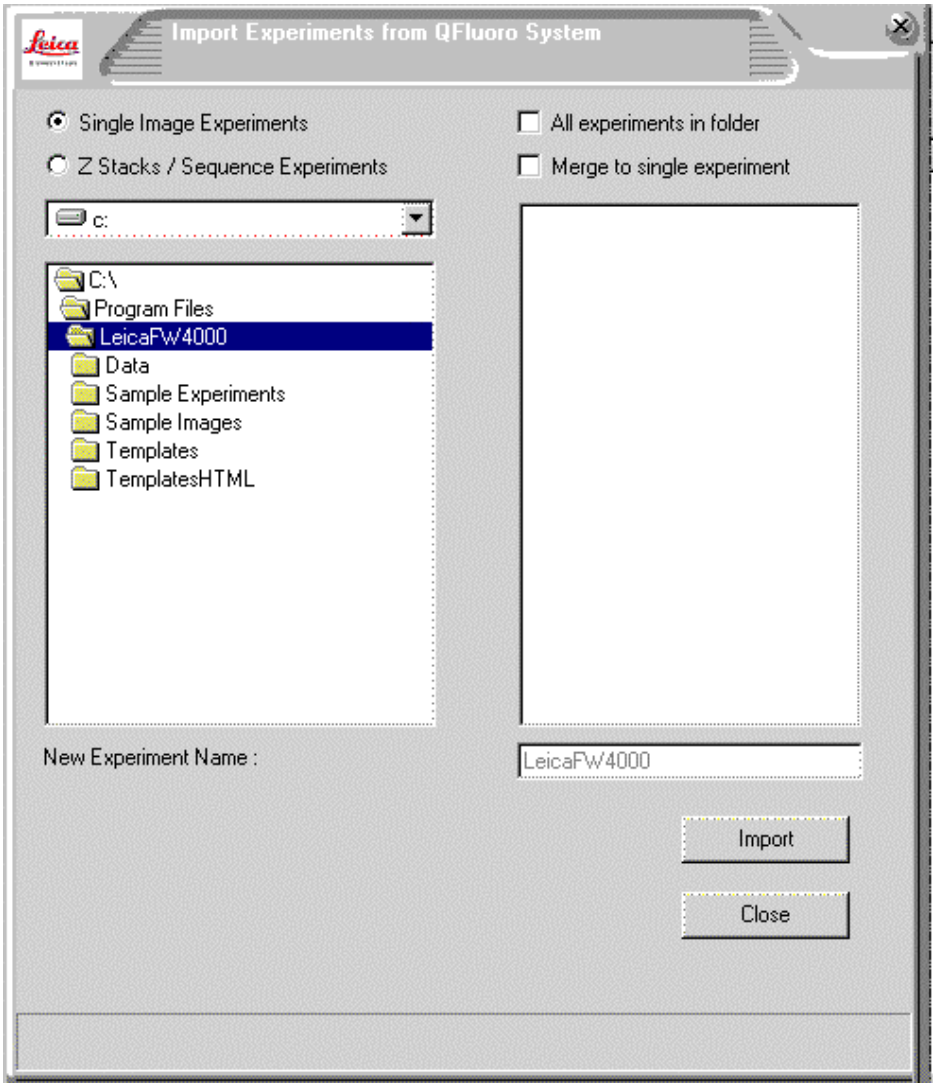
6. Select the desired experiment(s) and press Retrieve.

7. Close the dialog

Importing Experiments from QFluoro

Single experiments, Z-Stacks or time-lapse experiments acquired in **Leica QFluoro** may be imported into Leica FW4000. Once imported, the experiment may be treated as though it was acquired in **Leica FW4000** and a user may utilise all of the **Leica FW4000** features with the imported imagery.

➡ To import an experiment from QFluoro, click the **Import** button. The **Import Experiments from QFluoro System** dialog is displayed.



- To import an experiment from **Leica QFluoro**, browse for the required **Leica QFluoro** image or experiment and select either **Single Image Experiment** or **Z stacks / Sequence Experiments** from the **Import Experiments from QFluoro System** dialog.
- To import all images in a folder, check **All Experiments in Folder**. Each image file name will be imported as a separate experiment.
- To rename your experiment, do so at this stage and proceed to import the experiment/s by clicking **Import** in the **Import Experiments from QFluoro System** dialog.

Importing Tiff Images into Leica FW4000

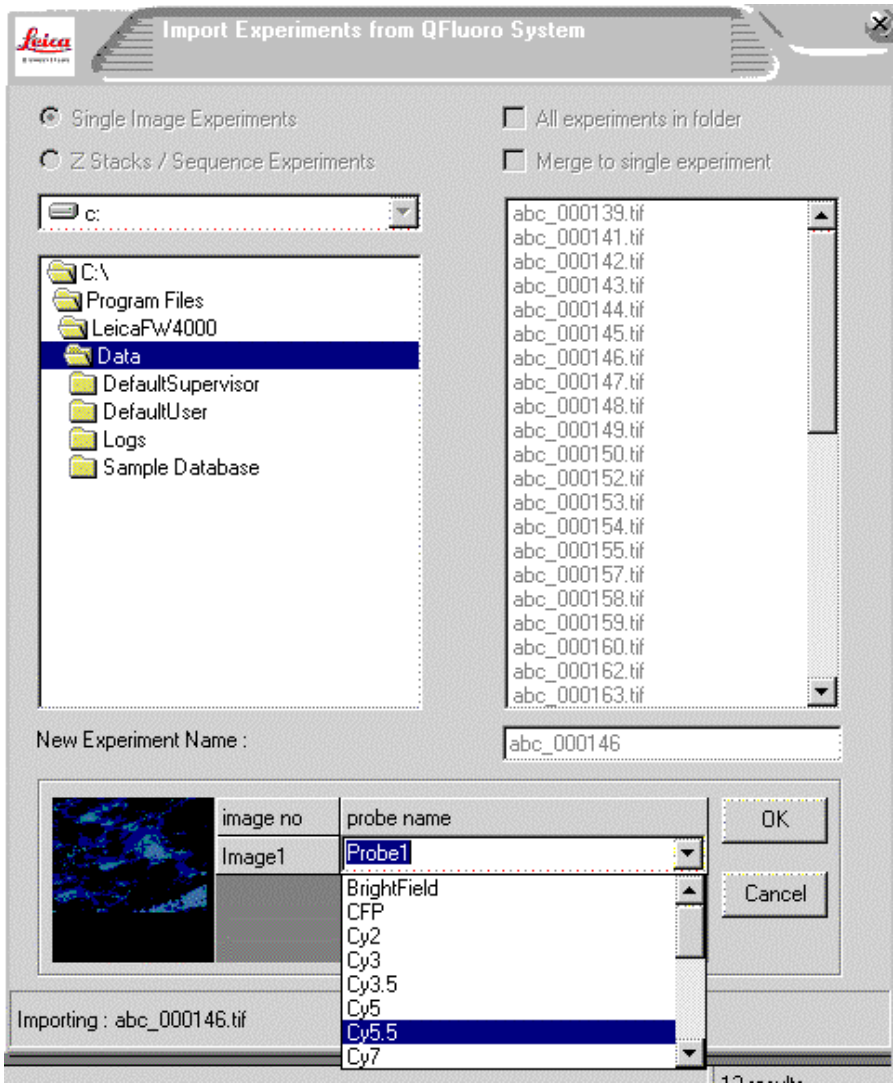
You may import monochrome and colour tiff images to **Leica FW4000** from another source. This allows you to import images not captured by **Leica FW4000** and to make an experiment of those images that may be loaded. You can process and measure the images imported into the experiment using **Leica FW4000** tools.

Composite images, generated in a third party piece of software, may be viewed during the import step, but if imported they may not be used for image processing.

Note: If you attempt to import .tiff images from **Leica QFluoro** that have lost their accompanying .nfo file, they must be treated as third party .tiffs, that is, each image must be assigned as a probe.

To import .tiff files:

1. Browse for the location of the images you wish to import in the **Import Experiments from QFluoro System** dialog. The tiff images will appear in the window on the right.
2. Select the images in the list that you wish to import, checking all experiments in the folder if you wish to automatically select all. Use the shift key to select all images between selected items, or the control key to select freely from the list.
3. Click **Import** and a preview of the first image is shown.



4. For each image imported, click on the probe name and a drop-down arrow will appear, from which you may select the probe name you wish to use for the imported image.

Note: The image size displayed in **Leica FW4000** will be taken from the first image imported.

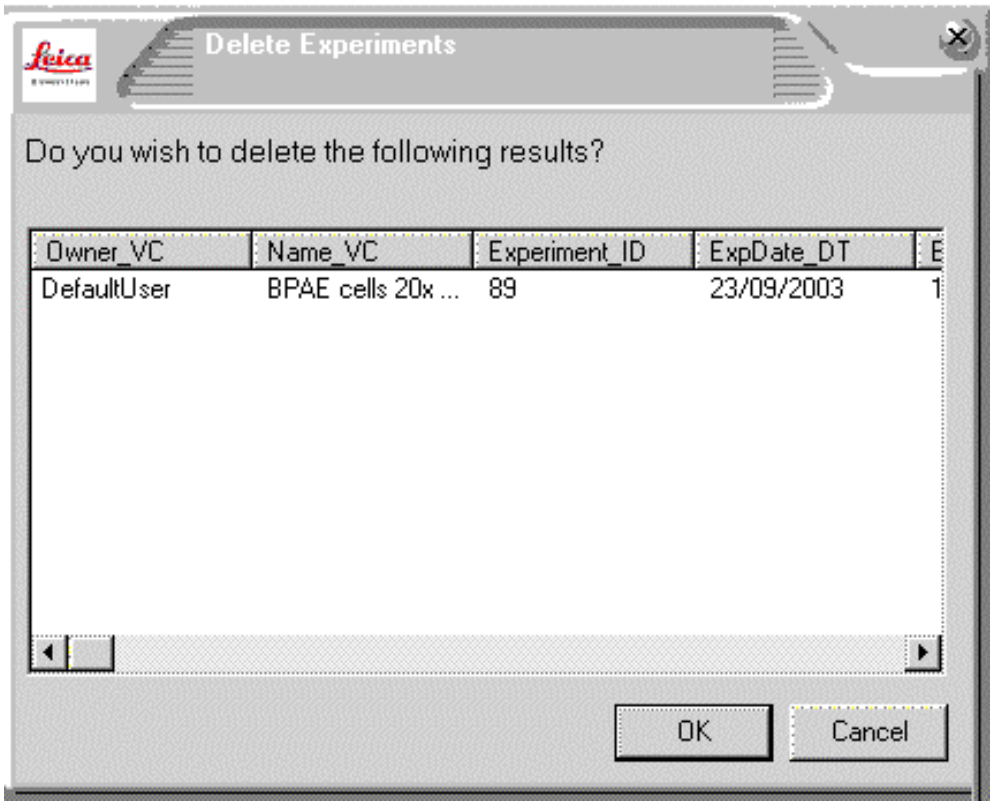
Note: Composite images generated in third party software, may be reviewed here but can be rejected from the import by selecting **IGNORE** from the list of probes.

Note: If the images imported into one experiment are of different dimensions, you are not able to generate a composite image from them in **Leica FW4000**.

Deleting Experiments

To delete an experiment:

1. Select one or more experiments, which you want to delete in the archive view, or one or more archived experiments to delete in the Retrieve view.
2. Click the **Delete** button. The **Delete Experiments** dialog is displayed.



3. Click **OK** to delete the experiment, or click **Cancel** to cancel the operation.

Note: In Retrieve mode, the archive files are deleted immediately. In Archive mode, experiments are flagged for deletion but not deleted immediately. When you close the Archive form, you must confirm that you still want to delete the experiments.

Click the Undo icon to undo the most recent set of deletes.